UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: July 17, 2014

Subject: Paraquat Dichloride. Review of toxicity studies

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Decision No.: 480431, 475176, 452219	**Registration No.:** GDCI-0661601-1172, RegRev-0262-1
Petition No.: NA	**Regulatory Action:**
Risk Assessment Type: NA	**Case No.:** NA
TXR No.: 0056764	**CAS No.:** 1910-42-5
MRID No.: 49122304, 49009501 – 49009508, 48877203	**40 CFR:** NA

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I. CONCLUSIONS:

None of the studies submitted and reviewed impact the hazard characterization of paraquat dichloride. With the exception of the acute inhalation toxicity study (MRID 48877203), the submitted studies are all non-guideline exploratory studies. The DERs are attached to this memo.

II. ACTION REQUESTED

Review voluntary submissions of paraquat dichloride toxicity studies

III. BACKGROUND

Studies in MRIDs 49009501 – 49009508 address developmental toxicity of paraquat in rabbits. An acceptable developmental toxicity study in rabbits is not available. However, HASPOC has waived the requirement for this study (TXR 0056294).

IV. RESULTS/DISCUSSION

1. Rattray, N. (2005) Paraquat dichloride technical material -- 4-hour acute inhalation toxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number HR2533-REG, December 1, 2005. MRID 48877203.

In an acute inhalation toxicity study (MRID 48877203), Paraquat dichloride technical material [33.4% w/w Paraquat cation (by HPLC); 46.1% Paraquat dichloride (by calculation); Batch #P51] was administered to groups of five male and/or female young adult Alpk:AP$_f$SD (Wistar derived) rats by dynamic nose-only exposure for four hours at target Paraquat ion concentrations of 0.4 µg/L (both sexes group1), 1.5 µg/L (females only, group 2), or 2.5 µg /L (females only, group 3). Animals were observed for 14 days after exposure.

Based on analytical results, mean calculated formulation concentrations during exposure were 1.08, 4.72, and 7.45 µg/L. The respective mean mass median aerodynamic diameters (MMADs) were 2.03 µm, 1.67 µm, and 1.06 µm and mean geometric standard deviation (GSDs) were 2.37, 1.62, and 1.84 for the low through high dose groups, respectively.

Mortalities included all group 3 animals (four deaths and one sacrifice on day 2) and one group 2 animal (sacrificed on day 10); all group 1 animals survived to study termination. Abnormal clinical signs during exposure included the following: wet fur and staining around the snout in all exposure groups; salivation in groups 1 and 2; and abnormal respiration (decreased rate and increased depth), decreased response to sound, and/or chromodacryorrhea in group 3. In group 1, post-exposure clinical signs included chromodacryorrhea on day 1 (in two animals) and a finding described as "whistling" in all of the animals on days 3-5 or 3-6. In group 2, post-exposure clinical signs included chromodacryorrhea on day 1 (one animal), and all of the animals exhibited abnormal respiratory noise consistent with respiratory tract irritation beginning on days 1-3 and continuing through day 10 or 12. All group 2 animals exhibited abnormal breathing depth and rate (increased or decreased) and hunched posture beginning on day 3 and resolving by day 6, except one continued to have hunched posture through unscheduled sacrifice on day 10. Other abnormal signs in this latter animal included piloerection and sides pinched in (days 4-10), staining around the mouth (days 6-9) and vaginal bleeding (day 10). Group 3 animals exhibited decreased activity, abnormal breathing rate and depth, hunched posture, "cold," abnormal respiratory noise, and/or sides pinched in on days 1 and/or 2 prior to death or sacrifice. Group 1 animals generally gained weight during both weeks of the study. The exceptions were one group 1 male and two group 1 females that lost weight during the first week but did gain weight during the second week of the study; of these, one female had a terminal body weight lower than her initial body weight, while the other two had a net gain across the entire study. All surviving group 2 animals had a net weight gain across the entire study; two did lose weight during the first week, and a third maintained the same weight during days 8-15. At necropsy, the group 2 animal that was sacrificed early had mottling and

depressed areas in the lungs, and the surviving group 2 animals had pale spots or areas in the lung. No other abnormal gross necropsy findings were recorded.

Four-hour Inhalation LC_{50} in female rats is > 4.2 µg /L and < 7.1 µg /L µg /L Paraquat dichloride technical
(> 0.36 µg /L and < 2.49 µg /L Paraquat ion)

Based on the four-hour inhalation exposure LC_{50}, Paraquat dichloride technical material is of HIGH Toxicity and is classified in EPA Toxicity Category I.

This study is classified as **Acceptable/Guideline**. It does satisfy the guideline requirements for an acute inhalation study (OCSPP 870.1300; OECD 403) in the rat.

2. Moxon, M. (1999) Paraquat dichloride - second dose range finding study in the rabbit (final report). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2965, August 6, 1999. MRID 49009508. Unpublished. 35 pages.

Moxon, M. (1999) Paraquat dichloride - dose range finding study in the rabbit (final report). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2964, August 6, 1999. MRID 49009507.

In a non-guideline range-finding feeding study (MRID 49009508), Paraquat dichloride technical (32.32% a.i.; Batch no. D9485/072) was administered to six female New Zealand White rabbits/group in feed at dietary concentrations of 228 ppm or 317 ppm for thirteen days, from day 1 through scheduled sacrifice and necropsy on day 14 (no vehicle control group included). The dietary concentrations were intended to result in dose levels of 8 and 12 mg/kg bw/day, respectively. Dose selection was based on a preliminary range-finding study (MRID 49009507). The purpose of the study was, in part, to determine a suitable highest dietary concentration level for use in a future range-finding study in pregnant rabbits.

The actual mean (±SD) doses received by the 228 and 317 ppm rabbits were 6.3±3.7 mg/kg bw/day (individual values: 0-14.9 mg/kg bw/day) and 4.8±3.6 mg/kg bw/day (individual values: 0-14.0 mg/kg bw/day), respectively.

There were no deaths. Treatment-related clinical signs consisted of "few feces on tray" in three 228 ppm females and five 317 ppm females (9 and 18 observations, respectively) and "no feces on tray" in one 228 ppm female and four 317 ppm females (1 and 7 observations, respectively). Relative to their respective absolute body weights on day 1, the 228 ppm group lost weight during days 1-6 (-7%), and the 317 ppm females lost weight during days 1-10 (-12%). Both groups showed some recovery but still had net mean body weight losses on day 14 (5% and 11% of day 1 body weight, respectively). Relative to day 1, both groups had decreased food consumption beginning on day 1, which reached its lowest level at about day 3. Some recovery was seen starting on approximately day 6 (for the 228 ppm group) or day 8 (for the 317 ppm group), although all of the values for the 317 ppm group and most of the

values for the 228 ppm group remained below the day 1 level. Mean food consumption of the 317 ppm group was lower than that of the 228 ppm group during days 2-13. It is unknown whether the effects on food consumption were direct effects or secondary to altered palatability of the diet due to the inclusion of the paraquat dichloride.

Because of the considerable variation in the actual dosage to the animals across the study duration, the results of this study cannot be used to establish a lowest-observed-adverse-effect level (LOAEL).

This range-finding feeding study in the rabbit is classified **Unacceptable/Non-guideline** because of the considerable variation in the actual dosage to the animals and the fact that the study was not conducted in compliance with GLP principles, including Quality Assurance. No vehicle control group was included.

3. Tinston, D. (1991) Paraquat - second teratogenicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2763, September 26, 1991. MRID 49009505. Unpublished.

Hodge, M. (1990) Paraquat - embryotoxicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/L/3423, October 18, 1990. MRID 49009502. Unpublished.

Tinston, D. and J. Barber (1991) Paraquat - second embryotoxicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2730, July 11, 1991. MRID 49009503. Unpublished.

Tinston, D. (1991) Paraquat - teratogenicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2749, September 26, 1991. MRID 49009504. Unpublished.

In a developmental toxicity study (MRID 49009505), paraquat (33.6% w/w paraquat ion; Lot/batch #YF6219 [ex No. 9 Product Stock Tank]) was administered to twenty inseminated female New Zealand White rabbits/dose by gavage in deionized water at dose levels of 0, 1.0, 1.5, or 2.0 mg/kg bw/day (dose volume 1 mL/kg bw) on gestation days (GDs) 7 through 19, inclusive, with the day of insemination designated as GD 1. On GD 30, surviving does were sacrificed and necropsied. Gravid uterine weights, corpora lutea counts, and the numbers and positions of live fetuses and early and late intrauterine deaths were recorded. Fetuses were weighed, examined for external anomalies (including cleft palate), sexed internally, subjected to visceral examination, and fixed in methanol. Following 24 hours fixation, the head of each fetus was cut along the fronto-parietal suture line, and the brain was examined, with the carcass subsequently processed for and subjected to skeletal examination. This study repeated a previous study (MRID 49009504) for which dose selection was based on two preliminary studies (MRIDs 49009502 and 49009503). [See Appendices I, II, and III of this DER for summaries.]

Mortality occurred in all treated groups, with 20, 17, 12, and 9 animals surviving to scheduled termination in the 0-, 1.0, 1.5, and 2.0 mg/kg groups, respectively. Two 2.0 mg/kg animals were found dead (GDs 21 and 22), and two, four, and four animals from the 1.0 , 1.5 , and 2.0 mg/kg groups, respectively, were killed between GDs 12 and 24 because of excessive weight loss and/or poor clinical condition. In addition, one, four, and five animals in the 1.0, 1.5, and 2.0 mg/kg groups, respectively, were killed following abortions (GDs 28, 20-29, and 21-30, respectively). There were no mortalities or abortions in the control group. Treatment-related clinical signs included few or no feces, thin appearance, and abnormal feces (diarrhea, signs of diarrhea, or mucus or blood). Significant ($p<0.05$ or 0.01) dose- and treatment-related mean body weight losses were seen at all dose levels during GD 7-19 (BW changes: +201.4 g, -98.0 g, -194.3 g, and -363.8 g in ascending dose order). There were correlated statistically significant treatment-related decreases in food consumption in 1.0 mg/kg animals during GD 10-16 (33-34% less than controls) and in 1.5 and 2.0 mg/kg animals throughout treatment (38-49% and 46-67% less than controls, respectively). These effects were most pronounced in animals that aborted, had total litter resorptions, or died/were sacrificed intercurrently. Potentially treatment-related gross findings were noted among the animals that were found dead or sacrificed ahead of schedule. These included stomach lesions such as ulceration of the glandular or non-glandular portion, pale or hemorrhagic areas, distension, abnormal contents, and/or thin wall (in 2/3, 4/8, and 7/11 animals) and fluid colon contents (in 1/3, 2/8, and 4/11 animals). **Under the conditions of this study, the maternal lowest-observed-adverse-effect level (LOAEL) for paraquat dichloride in rabbits dosed on days 7-19 of gestation (with day of insemination = GD 1) is 1.0 mg/kg bw/day, based on death, abortion, weight loss, decreased food consumption, and gross pathology of the stomach and colon. The maternal NOAEL is not identified.**

Relative to controls, all treated groups had lower mean numbers of corpora lutea (11.47, 10.18, 10.44, and 9.63 for 0-, 1.0, 1.5, and 2.0 mg/kg groups, respectively). This, in conjunction with higher mean percentages preimplantation loss (8.3%, 15.3%, 20.9%, and 18.3%) resulted in lower mean numbers of implantations (10.53, 8.73, 8.56, 7.88; $p<0.05$) and lower mean numbers of live fetuses (9.59, 8.18, 6.00, and 7.38; $p<0.05$ at 1.5 and 2.0 mg/kg bw/day). The higher percentages or preimplantation loss in the treated groups and associated decreases in mean numbers of implantations and live fetuses may be treatment-related, reflecting very early resorptions (i.e. on GDs 7-8); however, it is also possible that the differences are related to animal husbandry issue(s), insemination technique, or compromised animal health. The 1.5 mg/kg group had an increased mean percentage postimplantation loss (20.9% vs. 8.1% for controls; $p<0.01$) that was associated with an increased proportion of dams with at least one early intrauterine death (55.6% vs. 5.9% of controls). Two total litter resorptions (one in each of the 1.0 and 1.5 mg/kg groups) were considered treatment-related. Mean fetal weight and fetal sex ratio were not affected by treatment.

The total numbers of fetuses (and litters) evaluated in the control, 1.0, 1.5, and 2.0 mg/kg groups were 163 (17), 90 (11), 54 (9), and 59 (8), respectively. In these same respective groups, major defects were observed in a total of 3 (3), 3 (3), 3 (3), and 2 (1) fetuses (and litters). These included major head defects in 1 (1) and 2 (1) 1.5 and 2.0 mg/kg fetuses (and

litters), respectively, limb defects in 1 (1) and 3 (3) 1.0 and 1.5 mg/kg fetuses (and litters), respectively, and spina bifida occulta in one 1.0 mg/kg fetus. At 2.0 mg/kg bw/day there were significant ($p<0.05$) treatment-related increases in the litter incidences of "27 pre-sacral vertebrae" and "27 pre-sacral vertebrae with any extra 13th rib" (100% vs. 52.9% of control litters for both), and the incidence of "any extra 13th rib" was significantly increased at all treatment levels (50.9%, 68.9%, 72.2%, and 79.7% of fetuses with >88% of litters affected in all groups). Although treatment-related, the increased incidences of variants are not considered adverse. **The developmental LOAEL for paraquat dichloride in rabbits dosed on days 7-19 of gestation (with day of insemination = GD 1) is 1.0 mg/kg bw/day, based on abortion and total litter resorption. The developmental NOAEL is not identified.**

In light of the fact that the disparity between the results of this study and MRID 49009504 calls into question the conduct of the study, and because the study was not conducted in compliance with the Principles of Good Laboratory Practice, no Quality Assurance statement was provided, and no individual data were provided, this developmental toxicity study in the rabbit is classified **Unacceptable/Guideline** and *does not satisfy* the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) in the rabbit.

4. Farnworth, M., Foster, J., and Lock, E. (1993). Paraquat—The toxicity of paraquat to rabbits following oral administration—Final Report. Zeneca Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, United Kingdom. CTL Study Numbers: XB2434, XB2567, XB2607, and XB2610. Report Number CTL/R/1164. September 20, 1993. MRID 49009501.

In an acute oral toxicity/Median Lethal Dose study (MRID 49009501), paraquat dichloride (33.0% paraquat ion (w/w) in stock solution, batch YF6219,) was administered by gavage to groups of 2 female New Zealand rabbits at dose levels of 2, 4, 8, 12, 16, 20, 24, or 30 mg paraquat ion/kg bw, or to groups of 4 female New Zealand rabbits at dose levels of 40 or 50 mg paraquat ion/kg bw. Animals were observed for up to 10 days unless they had been sacrificed before scheduled termination. In two Tissue Distribution and Excretion experiments [^{14}C]-methyl labeled paraquat dichloride (33.0% paraquat ion (w/w) in stock solution, (a mixture of unlabeled and radiolabeled paraquat) was administered by gavage to 20 female New Zealand rabbits at a dose level of 30 mg paraquat ion/kg bw, with 5 animals being sacrificed after 1, 4, 24, and 48 hours. In a third Tissue Distribution and Excretion experiment [^{14}C]-labeled paraquat dichloride was administered by gavage to groups of 4 female New Zealand rabbits at dose levels of 0, 2, or 30 mg paraquat ion/kg bw, with paraquat-treated animals being sacrificed after 144 and 72 hours at the lower and higher doses, respectively.

In the Median Lethal Dose study, rabbits receiving up to 12 mg paraquat ion/kg bw showed no signs of toxicity over the 10-day period of observation. Higher doses resulted in body weight loss, decreased food consumption and some hematuria. All four animals receiving a single oral dose of 50 mg paraquat ion/kg bw died during the observation period. It was concluded that the median lethal dose is between 40 and 50 mg paraquat ion/kg bw following a single oral treatment. Microscopic pathology of the kidneys of the animals with azotemia showed multifocal hydropic change in the S2 segment of the proximal tubules. The three

Tissue Distribution and Excretion experiments showed that the peak concentration in blood plasma was reached within one hour after treatment and that the concentration rapidly returns to near zero following treatment. No toxic effects were associated with the single dose of 2 mg paraquat ion/kg bw. At both doses (2 or 30 mg/kg), only about 10% of the oral dose was absorbed, and it was excreted in the urine. At the higher dose, both creatinine and urea levels in urine far exceeded the normal range at 2 and 3 days after treatment. While the lower dose of paraquat had no effect on urinary output, the higher dose reduced the urine flow by about 50% over the duration of the study and also produced a marked reduction in fecal output. As a result of the reduced urine and fecal outputs, only a small proportion of the administered dose was eliminated by these routes during the 72 hours study. At the oral dose of 30 mg paraquat ion/kg bw, there was both functional and morphological renal injury, which was thought to contribute to the mortality observed at higher doses. Microscopic pathology of the kidneys showed signs of multifocal hydropic change in the S_2 segment proximal tubules, interstitial fibrosis, multifocal tubular necrosis in the S_2 segment proximal tubules, tubular dilation, luminal casts, interstitial nephritis, and interstitial fibrosis. Histological examination of stomachs, duodenums, livers, and lungs of these same animals showed no compound-related effects, with the possible exception of one animal that, at 72 hours after dosing, had submucosal edema of the stomach wall, squamous metaplasia of the mucosa, and mucosa atrophy of the stomach. None of the 3 control animals showed any abnormalities in these organs in the microscopic pathology examination. There was no testing in this study of effects on hematology, most clinical chemistry parameters, organ weights, or gross and histologic pathology in most organs and tissues typically studied.

The LOAEL is a single oral dose of 30 mg paraquat ion/kg bw based on renal damage revealed by azotemia and by microscopic pathology findings of multifocal hydropic change in the S_2 segment of the proximal tubules and additional renal damage. No NOAEL is indicated because of the limited testing of only 2 animals at all but one of the lower doses tested. While no signs of toxicity were seen in any animals receiving single oral doses as high as 12 mg paraquat ion/kg bw, some animals at the doses of 16, 20, and 24 mg paraquat ion/kg bw exhibited loss of appetite, and one animal at the 24 mg paraquat ion/kg bw dose had unspecified loss of weight and hematuria on day 8 of observation. The data are considered too sparse at the doses of 16, 20, and 24 mg paraquat ion/kg bw to provide the basis for a LOAEL.

This acute oral toxicity/median lethal dose and tissue distribution and excretion study in the rabbit is **Acceptable (non-guideline)**. This non-guideline study provided useful information about the toxic effects of paraquat when administered orally as a single dose to female rabbits.

5. Beck, M.J. (2013) Subchronic (91-day) dietary study to assess the effects of paraquat dichloride on dopaminergic neurons in C57BL/6J mice. WIL Research Laboratories, LLC, 1407 George Rd., Ashland, OH 44805-8946. Laboratory report number: WIL-639158, January 24, 2013. MRID 49122304.

In a nonguideline subchronic neurotoxicity study (MRID 49122304) paraquat dichloride (99.9% a.i., batch/lot # ASJ10083-03 [WIL ID no. 110018]) was administered continuously

in the diet to 41 C57BL/6J mice/sex/group at dose levels of 0, 10 or 50 ppm (equivalent to 0, 1.7, and 10.2 mg/kg bw/day in males, respectively, and 0, 2.7, and 15.6 mg/kg bw/day in females, respectively) for 13 weeks. A positive control group of 31 C57BL/6J mice/sex was administered MPTP (l-methyl-4-phenyl-l,2,3,6-tetrahydropyridine hydrochloride), prepared in 0.9% physiological sterile saline, at 10 mg/kg bw/dose via 4 intraperitoneal (IP) injections spaced approximately 2 hours apart on a single day, 7 days prior to euthanasia, at a dose volume of 2.5 mL/kg/dose. Clinical examinations, body weights, and food consumption were recorded at selected intervals during the study. After 91, 92, 93, or 94 days of dietary exposure, 20 mice/sex/group designated for stereology assessments (Subset I) were anesthetized, perfused *in situ*, and the brains removed and shipped to Experimental Pathology Laboratories, Inc. for further processing and stereological analysis. After 4, 8 or 13 weeks of exposure, 5 mice/sex/time point from the 0, 10, or 50 ppm paraquat dichloride exposure groups and 5 mice/sex from the MPTP group at 13 weeks designated for pathological assessments (Subset II) were anesthetized, perfused *in situ*, and the brains removed, weighed and measured. The brains were shipped to NeuroScience Associates for sectioning and staining, and the slides were then shipped to Tox Path Specialists, LLC for neuropathological evaluation. After 95 days of exposure, 6 mice/sex/group designated for neurochemistry assessments (Subset III) were euthanized, the brain removed, and the striatum collected and shipped to RTI International for analysis of the concentrations of serotonin, dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA).

The analytical data indicated that the mixing procedure of the test diet was not adequate with % RSDs generally much greater than 10% (ranging from 8.9% to 68% RSD for the 10 ppm diet and 8.5% to 37% RSD for the 50 ppm diet). The variance between nominal and actual dosage to the animals was generally acceptable except for the 10 ppm diet formulations on the last sample day (150% of target); reanalysis revealed a concentration within 16% of target. Stability analysis revealed ending concentrations much greater than the starting concentrations. Although a previous validation study reported acceptable 12 day stability data, the results from this study were not acceptable.

Effects of treatment with paraquat dichloride were limited to transient, statistically significantly reduced body weight gain over the first week of treatment in males at 50 ppm (-83% of controls). Reductions in mean absolute body weight observed in males at 50 ppm were not biologically significant, being within 5% of the control values. Although the reduced body weight gain was transient, it was considered an adverse effect of treatment because it was not accompanied by a corresponding reduction in food consumption. No definitive, treatment-related effects on food consumption were observed during the study. Changes in mean absolute body weight and body weight gain in males at 10 ppm and females at 10 and 50 ppm were not considered biologically relevant. Treatment with 10 ppm or 50 ppm paraquat dichloride did not result in any observable clinical signs. Mortality of three males at 10 ppm and one male and one female at 50 ppm were not ascribed to treatment. No changes were noted during gross necropsy, and treatment with up to 50 ppm paraquat dichloride did not affect brain weight, brain measurements, stereological evaluation, neuropathological assessment of brain sections, or neurochemical concentrations of dopamine or its metabolites DOPAC and HVA in the striatal tissue of treated mice.

The response of the animals to the positive control was acceptable. Male and female mice treated with MPTP exhibited reversible clinical signs including hunched posture, piloerection, hypoactivity, and/or tremors following one or more dose administrations. These clinical signs were not present a week following dosing. In the week following dosing, males and females exhibited weight loss which was attributed to treatment with MPTP, but no definitive effects on food consumption were observed. Treatment with MPTP did not affect brain weights or measurements. A statistically significant decrease in tyrosine hydroxylase positive (TH^+) neurons and in the total contour volume was seen in male mice, but not female mice. In the striatal tissues from male mice treated with MPTP, marked decreases were observed in mean dopamine, DOPAC, and HVA concentrations with an associated increase in the mean dopamine turnover. The authors state that these findings are consistent with the known neurotoxicity of MPTP. The decreases in mean dopamine, DOPAC, and HVA concentrations observed for the striatal tissues obtained for the MPTP-treated female mice as compared to those for the control female mice were less than noted for the male mice.

Based on a transient decrease in body weight gain, the LOAEL was 50 ppm (10.2 mg/kg bw/day in males and 15.6 mg/kg bw/day in females), and the NOAEL was 10 ppm (1.7 mg/kg bw/day in males and 2.7 mg/kg bw/day in females).

The study is classified as **Unacceptable/ Non-Guideline**. The study is unacceptable because the analysis of the test diet formulations revealed unacceptable homogeneity and stability results. The study was not designed to meet guideline requirements.

6. Moxon, M. (1999) Paraquat dichloride - Dose range finding study in pregnant rabbits (final report). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2963, August 6, 1999. MRID 49009506.

In a non-guideline range-finding feeding study (MRID 49009506), paraquat dichloride technical (32.32% a.i.; Batch no. D9485/072) was administered to ten time-mated female New Zealand White rabbits/group in feed at fixed dietary concentrations of 0, 35, 70, or 140 ppm from gestation day 8 through gestation day 21 to achieve nominal doses of 0, 2, 4 and 8 mg paraquat dichloride/kg/day, respectively. However the actual dose equated to 1, 2 and 4mg/kg/day rather than 2, 4 and 8mg/kg/day. There was little variation in dose received during the study for the 35ppm group but, in the 70 and 140ppm groups, daily variation occurred with the changes in food consumption. Dose selection was based on preliminary range-finding studies (MRID 49009507, 49009508). The purpose of this study was to investigate the effects of dietary administration of paraquat dichloride on the pregnant New Zealand White rabbit and to determine appropriate dose levels for a developmental toxicity study. Previous developmental studies in rabbits (CTL Report Numbers CTL/P/2749 and CTL/P/2763 – not available for review) administering paraquat dichloride by oral gavage at dose levels of 1-2 mg/kg/day resulted in overt maternal and fetal toxicity which made it difficult to assess the developmental toxicity because of the low number of litters available for external examination.

Rabbits were examined daily for clinical conditions. Body weights and food consumption were recorded daily. Plasma creatinine and basal blood urea nitrogen were measured on day

18. The animals were terminated on day 30 of gestation. The animals were examined macroscopically. Number of corpora lutea, gravid uterus weight, implantations, live fetuses, early and late intra-uterine deaths and fetal weight were all recorded. Fetuses were examined for external abnormalities only.

Two animals from the 140 ppm group were sacrificed on day19 and another animal on Day 18 due to weight loss, negligible food consumption and few feces. Post mortem examination revealed abnormal stomach contents in 2 of the 3 animals. There was a slightly higher incidence of animals with reduced feces in the 70 and 140 ppm groups. No other treatment related clinical conditions were reported. Excluding the three animals from the 140 ppm group that were sacrificed prior to study termination, there was no effect on body weights or food consumption.

There was no difference in plasma creatine and basal blood nitrogen among groups except for the 140 ppm group where an increase in plasma creatine was seen when the 3 animals that were sacrificed prior to study termination were included in the calculation of the mean.

Three of the 10 animals in the 70 ppm group had abnormal stomach contents and 2 had a sloughed glandular mucosa. These findings were not seen in the animals given 140 ppm which survived to scheduled termination. However, one animal given 140 ppm did have areas of ulceration in the stomach (and 2 of the 3 animals which died during the study had abnormal stomach contents).

Three of the 10 animals in the 70 ppm group had abnormal stomach contents and 2 had a sloughed glandular mucosa. These findings were not seen in the animals given 140 ppm which survived to scheduled termination. However, one animal given 140 ppm did have areas of ulceration in the stomach (and 2 of the 3 animals which died during the study had abnormal stomach contents).

There was no evidence for an adverse effect of paraquat dichloride on the number, growth, external abnormalities or survival of the fetuses in utero.

The reviewer finds this range-finding study to be inconclusive. Actual dosage to the animals was variable. The diet was not analyzed for homogeneity or stability. No individual animal data was provided. The developmental segment of the study did not provide all parameters. A lowest observed adverse effect level (LOAEL) could not be established from this study. Equivocal reduction in body weight and food consumption was seen at the 70 and 140 ppm group animals along with reduced fecal excretion. Individual clinical signs, body weight, food consumption, cesarean section and gross pathology data were not provided. The study was not conducted in compliance with the GLP principles. This study is classified **Unacceptable/non-Guideline.**

DATA EVALUATION RECORD

PARAQUAT DICHLORIDE

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT;
OCSPP 870.1300 [§81 3]; OECD 403

MRID 48877203

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Science Info. Mgmt. Branch, Health Effects Division (7509P) **Date:** 7/17/14

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TXR#: 0056764

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat; OCSPP 870.1300 [§81-3]; OECD 403.

PC CODE: 061601 **DP BARCODES:** D404037

TEST MATERIAL (PURITY): Paraquat dichloride technical material [33.4% w/w paraquat cation (by HPLC); 46.1% paraquat dichloride (by calculation)]

SYNONYMS: none given.

CITATION: Rattray, N. (2005) Paraquat dichloride technical material -- 4-hour acute inhalation toxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number HR2533-REG, December 1, 2005. MRID 48877203. Unpublished. 102 pages.

SPONSOR: Syngenta Crop Protection, LLC, 410 Swing Road, Greensboro, North Carolina.

EXECUTIVE SUMMARY:

In an acute inhalation toxicity study (MRID 48877203), Paraquat dichloride technical material [33.4% w/w Paraquat cation (by HPLC); 46.1% Paraquat dichloride (by calculation); Batch #P51] was administered to groups of five male and/or female young adult Alpk:APfSD (Wistar derived) rats by dynamic nose-only exposure for four hours at target Paraquat ion concentrations of 0.4 µg/L (both sexes group1), 1.5 µg/L (females only, group 2), or 2.5 µg /L (females only, group 3). Animals were observed for 14 days after exposure.

Based on analytical results, mean calculated formulation concentrations during exposure were 1.08, 4.72, and 7.45 µg/L. The respective mean mass median aerodynamic diameters (MMADs) were 2.03 µm, 1.67 µm, and 1.06 µm and mean geometric standard deviation (GSDs) were 2.37, 1.62, and 1.84 for the low through high dose groups, respectively.

Mortalities included all group 3 animals (four deaths and one sacrifice on day 2) and one group 2 animal (sacrificed on day 10); all group 1 animals survived to study termination. Abnormal clinical signs during exposure included the following: wet fur and staining around the snout in all exposure groups; salivation in groups 1 and 2; and abnormal respiration (decreased rate and increased depth), decreased response to sound, and/or chromodacryorrhea in group 3. In group 1, post-exposure clinical signs included chromodacryorrhea on day 1 (in two animals) and a finding described as "whistling" in all of the animals on days 3-5 or 3-6. In group 2, post-exposure clinical signs included chromodacryorrhea on day 1 (one animal), and all of the

animals exhibited abnormal respiratory noise consistent with respiratory tract irritation beginning on days 1-3 and continuing through day 10 or 12. All group 2 animals exhibited abnormal breathing depth and rate (increased or decreased) and hunched posture beginning on day 3 and resolving by day 6, except one continued to have hunched posture through unscheduled sacrifice on day 10. Other abnormal signs in this latter animal included piloerection and sides pinched in (days 4-10), staining around the mouth (days 6-9) and vaginal bleeding (day 10). Group 3 animals exhibited decreased activity, abnormal breathing rate and depth, hunched posture, "cold," abnormal respiratory noise, and/or sides pinched in on days 1 and/or 2 prior to death or sacrifice. Group 1 animals generally gained weight during both weeks of the study. The exceptions were one group 1 male and two group 1 females that lost weight during the first week but did gain weight during the second week of the study; of these, one female had a terminal body weight lower than her initial body weight, while the other two had a net gain across the entire study. All surviving group 2 animals had a net weight gain across the entire study; two did lose weight during the first week, and a third maintained the same weight during days 8-15. At necropsy, the group 2 animal that was sacrificed early had mottling and depressed areas in the lungs, and the surviving group 2 animals had pale spots or areas in the lung. No other abnormal gross necropsy findings were recorded.

Four-hour Inhalation LC_{50} in female rats is > 4.2 µg /L and < 7.1 µg /L µg /L Paraquat dichloride technical
(> 0.36 µg /L and < 2.49 µg /L Paraquat ion)

Based on the four-hour inhalation exposure LC_{50}, Paraquat dichloride technical material is of HIGH Toxicity and is classified in EPA Toxicity Category I.

This study is classified as **Acceptable/Guideline**. It does satisfy the guideline requirements for an acute inhalation study (OCSPP 870.1300; OECD 403) in the rat.

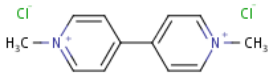
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Paraquat dichloride technical

Description:	Brown liquid
Batch #:	P51
Purity:	33.4% w/w paraquat cation (by HPLC); 46.1% paraquat dichloride (by calculation)
Compound stability:	Expiration Date: January 2008; Stored at ambient temperature in the dark
CAS # of TGAI:	1910-42-5
Structure:	



2. Vehicle: Filtered, dry air was used in the generation of the test atmosphere. Formulation in deionized water.

3. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar derived)
Age/weight at study initiation:	Group 1 males: 8-9 weeks/346-381 g; Group 1 females: 8-9 weeks/246-271 g; Group 2 females: 7-8 weeks/217-228 g; Group 3 females: 14-15 weeks/256-325.
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire, U.K.
Housing:	In same-sex groups of five, in multiple rat racks
Diet:	RM1 Diet (supplied by Special Diets Limited, Witham, Essex, U.K.), *ad libitum* except during exposure
Water:	Municipal tap water, *ad libitum* except during exposure
Environmental conditions:	**Temperature:** 22±3°C **Humidity:** 30-70% **Air changes:** ≥15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	At least five days.

B. STUDY DESIGN:

1. In life dates: Start: May 10, 2005; End: October 25, 2005.

2. Animal assignment: An unspecified method was used to assign the animals to the test groups noted in Table 1. At the time of exposure, individual body weights were within ±20% of the group mean for the appropriate sex.

TABLE 1: Study design								
Group	**Target paraquat ion conc. (µg/L)**	**Measured particulate concentration µg/L**	**Mean analytical paraquat ion conc. (µg/L)**	**Calculated paraquat dichloride technical material conc. (µg/L)**	**MMAD (µm)**	**GSD**	**Mortality**	
							Males	**Females**
1	**0.4 (Group 1)**	0.73±0.29	0.36	1.08	1.74-2.32	2.36-2.38	0/5	0/5
2	**1.5 (Group 2)**	3.23±0.13	1.58	4.72	1.37-1.97	1.56-1.67	--	1/5
3	**2.5 (Group 3)**	9.0±2.7	2.49	7.45	0.96-1.15	1.78-1.90	--	5/5

Data taken from p. 12, 20 MRID 48877203.

3. **Dose selection rationale:** The exposure concentrations were selected based on the known inhalation toxicity of paraquat. Exposure concentrations of 0.4 and 1.5 µg paraquat ion/L were used initially, and a further concentration of 2.5 µg paraquat ion/L (group 3) was selected for use to enable the calculation of an LC_{50}. Female rats were exposed to all concentrations, and male rats were exposed only to the lowest concentration in order to verify that males did not exhibit greater sensitivity than females.

4. **Generation of the test atmosphere / chamber description:** Chamber conditions are given in Table 2. The test atmospheres were generated using a peristaltic pump to meter the test material to a glass concentric-jet atomizer supplied with dried, filtered air. The atmospheres were carried directly to the exposure chamber without the use of diluting air at a flow rate to achieve a minimum of 12 air changes per hour. The circular, nose-only exposure chamber consisted of two sections of Perspex tubing drilled with equidistant 28-mm-diameter holes, into which the polycarbonate restraining tubes were pushed to give a good seal. Air flows were monitored continuously using variable area flowmeters (KDG Flowmeters, Burgess Hill, Sussex, UK) and were altered as necessary to maintain the target concentration. Temperature and relative humidity inside the chamber were measured using a portable digital thermometer and a relative humidity monitor. All three parameters were recorded 9, 7 and 9 times during exposure, for groups 1, 2, and 3 respectively.

Table 2: Chamber conditions.			
Calculated Formulation Conc. (µg/L) (Mean ± S.D [Range])	1.08±0.46 [0.46-1.65]	4.72±1.63 [1.53-5.91]	7.45±3.88 [3.53-12.60]
Chamber Volume (L)	27.6		
Total Airflow (L/min)	15-16		
Temperature (° C)	20.2-21.8	20.4-20.6	19.8-20.0
Relative Humidity (%)	14-18	25-28	29-35
T_{99} (minutes)	5	8	8.5

Data taken from pp. 16, 19, 26-28, MRID 48877203.

Test atmosphere concentration: Samples for gravimetric analysis of the particulate concentration were collected from "close to" the breathing zone of the animals at six or eight intervals during each exposure by drawing a known volume of the test atmosphere through preweighed 25-mm A/E glass fiber filters (GLA) housed in a Delrin open-faced filter holder. The material collected on the filters and on the disassembled stages of the impactor (see below) was analyzed using high performance liquid chromatography (HPLC) with detection at 255 nm to determine the concentration of the paraquat ion. The concentration of the total formulation in the atmospheres was calculated based on these data and the percentage of paraquat ion in the formulation. Results are in table 1 above.

Particle size determination: Twice during each exposure, samples were withdrawn from the breathing zone of the animals using a Marple cascade impactor. The disassembled filters and impactor stages were weighed, and the amount of aerosol on each stage of the impactor was calculated based on analytical determination of the concentration of paraquat ion. The data were analyzed using log/probit transformation and linear regression to determine the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). Results are in table 1 above.

5. **Statistics:** For male and female data, the median lethal concentration and slope of the regression line were estimated by logistic regression. The study report stated that confidence limits were calculated using a likelihood ratio interval (Williams 1986). This was not done, however, due to the nature of the data that were attained (only one of three groups with a partial response).

C. METHODS:

1. **Clinical observations:** Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed frequently during exposure, and each rat was given a detailed clinical examination at the end of the 4-hour exposure. Detailed clinical observations were conducted daily during the 14-day observation period.

2. **Body weight:** Animals were weighed on days -1, 1, 8, and 15.

3. **Sacrifice and pathology:** Terminal sacrifices and any unscheduled sacrifices during the study were done via overdose of halothane anesthesia. All animals that died or sacrificed early and those sacrificed on schedule were subjected to gross pathological examination.

II. RESULTS:

A. OBSERVATIONS:

1. **Mortality:** All of the group 1 animals survived to study termination. One group 2 female was killed on day 10 due to clinical signs. In group 3 on day 2, four animals were found dead and one was killed due to clinical signs.

2. **Clinical signs of toxicity:**

Group 1: During exposure (day 1), all of the animals had wet fur and staining around the snout, and some exhibited salivation. One male and one female had chromodacryorrhea on day 1 following exposure. All of the animals had a finding described as “whistling” on days 3-5 or 3-6 and were normal thereafter.

Group 2: During exposure, all of the animals had wet fur, staining around the snout, and salivation. On day 1 following exposure, one animal had chromodacryorrhea. All of the animals exhibited an abnormal respiratory noise consistent with respiratory tract irritation beginning on days 1-3 and continuing through day 10 or 12. All of the animals exhibited abnormal breathing depth and rate (increased or decreased) and hunched posture beginning

on day 3 and resolving by day 6 with the exception of the animal that was killed ahead of scheduled sacrifice (Day 10), which continued to exhibit hunched posture through the day of sacrifice. Other abnormal signs in this latter animal included piloerection and sides pinched in (days 4-10), staining around the mouth (days 6-9) and vaginal bleeding (day 10).

Group 3: During exposure, all of the animals had wet fur, staining around the snout, abnormal respiration (decreased rate and increased depth), and decreased response to sound, and some also had chromodacryorrhea. Abnormal observations in these animals on days 1 and/or 2 included decreased activity, abnormal breathing rate and depth, hunched posture, cold, abnormal respiratory noise, and sides pinched in.

B. BODY WEIGHT AND WEIGHT GAIN: Most group 1 animals gained weight during both weeks of the study. The exceptions were one male and two females that lost weight during the first week but did gain weight during the second week of the study; of these, one female had a terminal body weight lower than her initial body weight, while the other two had a net gain across the entire study. All surviving group 2 animals had a net weight gain across the entire study; two did lose weight during the first week, and a third maintained the same weight during days 8-15.

C. GROSS PATHOLOGY: Reported abnormal findings were limited to mottling and depressed areas in the lungs of the group 2 animal that was sacrificed ahead of schedule and pale spots or areas in the lungs of the surviving group 2 animals.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author stated that the test atmospheres were acceptable with regard to their general stability and physical characteristics. The study author attributed the differences seen between the analyzed paraquat ion concentrations (expressed as a percentage of the total particulate) and the percent concentration of paraquat ion in the test material to the need to dilute the neat test material a considerable amount in order to achieve the very low atmospheric concentrations required for the study. This was why the study author expressed the target and achieved exposure concentrations in terms of analyzed paraquat ion. The study author concluded that the median lethal concentration of paraquat ion was 1.79 µg/L for females only (equivalent to 2.47 µg paraquat dichloride/L or 5.36 µg paraquat dichloride technical material/L).

B. REVIEWER COMMENTS: The reviewer is in agreement with the study author conclusions except for the LC_{50} value. Only one of three groups showed partial mortality response which makes it difficult to determine an actual LC_{50} value. It is more appropriate to estimate the LC_{50} value as greater 4.2 µg/L and less than 7.1 µg/L. Even without an exact LC_{50}, the results are sufficient for hazard evaluation and classification purposes. Based on the four-hour inhalation exposure LC_{50}, Paraquat dichloride technical material is of **HIGH Toxicity** and is classified in **EPA Toxicity Category I**. The study is classified as **Acceptable/Guideline** and does satisfy the guideline requirements for an acute inhalation study (OCSPP 870.1300; OECD 403) in the rat.

C. STUDY DEFICIENCIES: No deficiencies were identified.

DATA EVALUATION RECORD

PARAQUAT DICHLORIDE

STUDY TYPE: RANGE-FINDING FEEDING STUDY - RABBIT NON-GUIDELINE

MRIDs 49009507 and 49009508

Prepared for

Health Effects Division
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Prepared by
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Task 6-74

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Robert H. Ross, M.S., Program Manager	Signature: Robert H. Ross AE Date: 12/20/2013
Quality Assurance: Angela M. Edmonds, B.S.	Signature: Angela M. Edmonds Date: 12/20/2013

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** July 16, 2014
EPA Work Assignment Manager: Lori Brunsman **Signature:**
Science Info. Mgmt. Branch, Health Effects Division (7509P) **Date:** 7/17/14
Template version 09/11

TXR#: 0056764

DATA EVALUATION RECORD

STUDY TYPE: Range-Finding Feeding Study - Rabbit; Non-Guideline.

PC CODE: 061601 **DP BARCODES:** D409213

TEST MATERIAL (PURITY): Paraquat dichloride technical (32.32% a.i.)

SYNONYMS: YF6219

CITATION: Moxon, M. (1999) Paraquat dichloride - second dose range finding study in the rabbit (final report). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2965, August 6, 1999. MRID 49009508. Unpublished. 35 pages.

Moxon, M. (1999) Paraquat dichloride - dose range finding study in the rabbit (final report). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2964, August 6, 1999. MRID 49009507. Unpublished. 35 pages.

SPONSOR: Zeneca Agrochemicals, Fernhurst, Haslemere, Surrey GU27 3JE. The submitter is Syngenta Crop Protection, LLC, 410 Swing Road, Greensboro, North Carolina.

EXECUTIVE SUMMARY:

In a non-guideline range-finding feeding study (MRID 49009508), Paraquat dichloride technical (32.32% a.i.; Batch no. D9485/072) was administered to six female New Zealand White rabbits/group in feed at dietary concentrations of 228 ppm or 317 ppm for thirteen days, from day 1 through scheduled sacrifice and necropsy on day 14 (no vehicle control group included). The dietary concentrations were intended to result in dose levels of 8 and 12 mg/kg bw/day, respectively. Dose selection was based on a preliminary range-finding study (MRID 49009507). The purpose of the study was, in part, to determine a suitable highest dietary concentration level for use in a future range-finding study in pregnant rabbits.

The actual mean (±SD) doses received by the 228 and 317 ppm rabbits were 6.3±3.7 mg/kg bw/day (individual values: 0-14.9 mg/kg bw/day) and 4.8±3.6 mg/kg bw/day (individual values: 0-14.0 mg/kg bw/day), respectively.

There were no deaths. Treatment-related clinical signs consisted of "few feces on tray" in three 228 ppm females and five 317 ppm females (9 and 18 observations, respectively) and "no feces on tray" in one 228 ppm female and four 317 ppm females (1 and 7 observations, respectively).

Relative to their respective absolute body weights on day 1, the 228 ppm group lost weight during days 1-6 (-7%), and the 317 ppm females lost weight during days 1-10 (-12%). Both groups showed some recovery but still had net mean body weight losses on day 14 (5% and 11% of day 1 body weight, respectively). Relative to day 1, both groups had decreased food consumption beginning on day 1, which reached its lowest level at about day 3. Some recovery was seen starting on approximately day 6 (for the 228 ppm group) or day 8 (for the 317 ppm group), although all of the values for the 317 ppm group and most of the values for the 228 ppm group remained below the day 1 level. Mean food consumption of the 317 ppm group was lower than that of the 228 ppm group during days 2-13. It is unknown whether the effects on food consumption were direct effects or secondary to altered palatability of the diet due to the inclusion of the paraquat dichloride.

Because of the considerable variation in the actual dosage to the animals across the study duration, the results of this study cannot be used to establish a lowest-observed-adverse-effect level (LOAEL).

This range-finding feeding study in the rabbit is classified **Unacceptable/Non-guideline** because of the considerable variation in the actual dosage to the animals and the fact that the study was not conducted in compliance with GLP principles, including Quality Assurance. No vehicle control group was included.

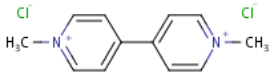
COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. According to the GLP Compliance Statement, the studies were not conducted in compliance with OECD Principles of Good Laboratory Practice (1997). Quality Assurance statements were not provided. Both studies included a Statement of Authentication signed by the study director that included the following assertion: The data described in this report have not been subjected to audit by the Laboratory's Quality Assurance Unit but are derived from a study which is considered to meet the principles of Good Laboratory Practice.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Paraquat dichloride

Description:	Technical material; dark brown liquid
Batch #:	D9485/072 [Bottle No. 128621]
Purity:	32.32% a.i.
Compound stability:	Confirmed by reanalysis after dosing had ended
CAS #of TGAI:	1910-42-5
Structure:	



2. Vehicle and/or positive control: The test material was incorporated into the diet; deionized water was used to help with mixing and pelleting. A positive control was not used in the study.

3. Test animals:

Species:	Rabbit (females, only)
Strain:	New Zealand White
Age/weight at study initiation:	Age not reported/body weights within a range of 3.3-4.0 kg
Source:	Harlan Interfauna Limited
Housing:	Individually in "mobile rabbit units"
Diet:	STANRAB SQC Standard Rabbit Diet (Special Diets Services Limited), *ad libitum*
Water:	Municipal tap water, *ad libitum*
Environmental conditions:	**Temperature:** 17±2°C **Humidity:** 40-70%, with an excursion to 83.5% on one occasion **Air changes:** 25-30/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	7 days

B. PROCEDURES AND STUDY DESIGN

1. Purpose: The purpose of this study was to investigate the effects of dietary administration of paraquat dichloride at nominal dose levels of 8 and 12 mg/kg bw/day to female New Zealand White rabbits over a 13 day treatment period in order to determine a suitable highest dietary concentration level for use in a future range-finding study in pregnant rabbits.

2. In life dates: Start: November 21, 1995; End: December 4, 1995.

3. **Animal Assignment:** Animal assignment is given in Table 1. The study report stated that the animals were randomized. No other details were provided.

TABLE 1. Animal assignment [a]

Group	Nominal dietary concentration (ppm)	Target dose (mg/kg bw/day)	Number assigned
1	228	8	6
2	317	12	6

[a] Data taken from text table, p. 14, MRID 49009508.

4. **Dose selection rationale:** Dose levels were selected based on the results from a range-finding study in female rabbits (MRID 49009507; summarized in Appendix) in which dietary administration of paraquat dichloride at concentrations of levels of 80, 179, and 332 ppm for up to 7 days resulted in dose-related effects on body weight gain and food consumption. See Appendix for more detail.

5. **Diet preparation and analysis:** A single batch of diet formulations was prepared on November 20, 1995. For each dietary formulation, a premix was made by dispensing the appropriate amount of paraquat dichloride (corrected for purity) with 12 mL of deionized water into the grinder bowl, using an additional 2 x 12 mL volumes of deionized water to wash the sample bottle into the grinder bowl, adding a 500-g quantity of the basal diet, and grinding the contents of the bowl until the test substance was thoroughly mixed into the diet, at which point the premix was passed through a 1.0 mm sieve, and any lumps were ground and sieved again. The premix was then transferred to a mixer and left to mix at minimum speed for 30 minutes. The premix was added to the rest of the control diet and mixed for 4 minutes using an automated mixer, and then was transferred with approximately 11% water to a different mixer and mixed for 6 minutes. The diet was then pelleted (3-mm size nominally), using a California pellet mill. The resultant pellets were dried in an autoclave at approximately 52°C for approximately 1 hour 45 minutes, cooled, and dispensed into color-coded plastic containers. The diets were stored at room temperature until used. Samples of treated food were analyzed for concentration on one occasion; the date this was done was not specified. Homogeneity and stability were not evaluated.

Results:
Concentration analysis: The mean measured concentrations of the 228 ppm and 317 ppm dietary formulations were within ±6% and ±11% of their respective nominal concentrations.

In the absence of stability and homogeneity data, it is unknown whether the mixing procedure was adequate or whether the variance between nominal and actual dosage to the study animals was acceptable.

C. METHODS:

1. **Mortality and clinical observations:** Animals were observed twice daily for mortality and morbidity. Detailed clinical observations were recorded daily and, where appropriate, at the same time that the animals were weighed.

2. **Body weight:** The animals were weighed daily, beginning on day -7.

3. **Food consumption:** Individual food consumption was recorded daily, beginning on day -7 (1 week prior to test diet administration).
4. **Test material intake:** Each animal's received dose was calculated daily as follows:

$$\text{Dose received (mg/kg bw/day)} = \frac{\text{dietary concentration (ppm) x food consumption (g)}}{\text{body weight (g) at the beginning of each period}}$$

5. **Sacrifice and pathology:** The animals were killed on day 14 via intravenous injection of pentoparbitone sodium solution. All were subjected to a gross necropsy involving external observation and examination of the thoracic and abdominal viscera.

D. DATA ANALYSIS:

1. **Statistical analyses:** Statistical analysis of the data was not performed.

2. **Historical control data:** Historical control data were not provided.

II. RESULTS:

A. MORTALITY AND CLINICAL OBSERVATIONS: No deaths were reported. Observations of "few feces on tray" and "no feces on tray" in both treated groups were attributed to treatment. "Few feces on tray" was exhibited by three 228 ppm females (9 observations) and five 317 ppm females (18 observations), and "no feces on tray" was exhibited by one 228 ppm female (1 observation) and four 317 ppm females (7 observations). Additional non-treatment-related clinical signs included sores or scabs and diarrhea.

B. BODY WEIGHT: Selected body weight data are given in Table 2. The mean weight losses in 228 ppm females during days 1-6 and in 317 ppm females during days 1-10 were attributed to treatment. Some partial recovery (resumption of mean body weight gain) was seen at both dietary concentrations, but both groups still had net mean body weight losses at the end of the study.

TABLE 2. Mean (±SD) body weight data (g) [a]

Parameter / Study day or interval		Dietary concentration in ppm [group size, n]	
		228 [n=6]	317 [n=6]
Absolute body weight:	Day -7	3652.5±162.4	3640.0±155.2
	Day 1	3889.7±244.8	3804.5±178.6
	Day 6	3628.8±253.1	3566.8±158.3
	Day 10	3634.0±164.5	3355.5±146.7
	Day 14	3696.8±180.0	3383.0±161.8
Body weight change [b]:	Days -7 to 1	237.2	164.5
	Days 1 to 6	-260.9	-237.7
	Days 6 to 10	5.2	-211.3
	Days 10 to 14	62.8	27.5
	Days 1 to 14	-192.9	-421.5

[a] Data taken from Table 4, pp. 25-26, MRID 49009508. [b] Calculated by reviewer using group mean values.

C. FOOD CONSUMPTION: Selected food consumption data are given in Table 3. Throughout the study, even prior to initiation of treatment, there was a lot of variability within the groups and from one day to the next with both groups showing a sharp decrease on day 3 and a sharp increase on day 9. Relative to day 1, food consumption of both groups decreased beginning on day 1 and reached its lowest level at about day 3. Some recovery was seen starting on approximately day 6 (for the 228 ppm group) or day 8 (for the 317 ppm group), although all of the values for the 317 ppm group and most of the values for the 228 ppm group remained below the day 1 level. Mean food consumption of the 317 ppm group was lower than that of the 228 ppm group during days 2-13.

TABLE 3. Mean (±SD) food consumption (g/animal/day) [a]

Study day	Dietary concentration in ppm [group size, n]	
	228 [n=6]	317 [n=6]
Day -7	99.2±32.6	78.8±36.8
Day -3	89.3±51.7	57.5±45.7
Day -1	144.5±84.6	137.8±59.8
Day 1	109.8±30.2	113.0±30.3
Day 5	63.0±50.0	37.8±30.5
Day 9	176.2±34.6	102.8±27.9
Day 13	162.3±41.5	63.5±44.7

[a] Data taken from Table 5, pp. 27-28, MRID 49009508.

D. COMPOUND INTAKE: Compound intake of the treated animals in mg/kg bw/day, as calculated by the reviewer using the individual data, is summarized in Table 4. Note: the daily means and ranges of individual values attained by the reviewer for 228 ppm animals on day 6 and for the 317 ppm animals on days 9 and 13 differ from those provided by the study author in the text (p. 16) and in Table 2, p.23.

TABLE 4. Paraquat dichloride doses (mg/kg bw/day) [a]

Study day or Parameter	Dietary concentration in ppm [group size, n]	
	228 [n=6]	317 [n=6]
Day 1	6.4±1.8	9.5±2.6
Day 2	5.7±4.0	4.2±3.6
Day 3	3.5±2.1	2.5±1.7
Day 4	2.8±2.5	3.8±2.2
Day 5	3.8±2.9	3.3±2.6
Day 6	3.1±2.3	2.9±2.6
Day 7	4.0±2.4	2.7±2.4
Day 8	5.5±3.1	3.2±3.0
Day 9	11.1±2.2	9.4±2.6
Day 10	8.8±2.8	4.2±3.3
Day 11	7.4±2.9	4.9±3.2
Day 12	9.3±1.1	6.3±4.7
Day 13	10.1±2.7	6.0±4.2
Overall mean	6.3±3.7	4.8±3.6
Range of individual values	0-14.9	0-14.0

[a] Data derived from Appendix D, p. 35, MRID 49009508. Values are Mean ± SD, where appropriate.

4. Gross pathology: The only abnormal finding was non-treatment-related: an accessory spleen in one 317 ppm female.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author noted that the nominal dose levels were not achieved because of the reduction in food intake and further noted that the two groups received similar doses, with the overall mean value being slightly higher in the 228 ppm group than in the 317 ppm group. The study author concluded that treatment with paraquat dichloride affected bodyweight and food consumption and that the decreased food consumption resulted in reduced fecal output. The study author stated that it was unclear whether the effects on food consumption and hence bodyweight and dose received were direct effects or secondary to altered palatability of the diet due to the inclusion of the paraquat dichloride. The study author noted that the similarity of the achieved doses and the high intra- and inter-animal variation in the dose level received on any one day made it impossible to interpret the data in terms of a dose relationship. Therefore, if the effects were due to a direct effect of paraquat dichloride, then the actual dose level producing them was uncertain. The study author also concluded that the study did not successfully accomplish its purpose of evaluating the effects in the rabbit of nominal dose levels of 8 and 12 mg paraquat dichloride/kg bw/day via incorporation of paraquat dichloride into the diet at fixed concentrations.

B. REVIEWER COMMENTS: The reviewer is in agreement with the study author and also notes that the usefulness of the results of the study is further limited by the absence of a vehicle control group and the small group size.

Because of the considerable variation in the actual dosage to the animals across the study duration, the results of this study cannot be used to establish a lowest-observed-adverse-effect level (LOAEL).

Because of the considerable variation in the actual dosage to the animals, the lack of homogeneity evaluation of the diet formulations, and the study was not conducted in compliance with GLP principles, including Quality Assurance, this non-guideline study is classified as **Unacceptable.**

C. STUDY DEFICIENCIES: The following deficiencies were noted:

- The study was not conducted in compliance with the Principles of Good Laboratory Practice.
- No Quality Assurance statement was provided.
- Individual clinical signs, body weight, food consumption, and gross pathology data were not provided.
- A vehicle control group was not included in the study.
- Stability and homogeneity of the prepared diet formulations were not evaluated.
- When the reviewer calculated daily means and standard deviations using the individual compound intake data the mean daily values for the 228 ppm animals on day 6 and for the 317 ppm animals on days 9 and 13 and the range of individual values for the 317 ppm animals differed from those provided by the study author in the text (p. 16) and in Table 2, p.23. It is unknown whether the discrepancies were due to typographical errors, exclusion of "outliers" by the study author, differences in rounding, or something else.

APPENDIX: Prenatal Developmental Toxicity Study - Rabbit; Range-finding

TEST MATERIAL (PURITY): Paraquat dichloride technical (32.32% a.i.)

CITATION: Moxon, M. (1999) Paraquat dichloride - dose range finding study in the rabbit (final report). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2964, August 6, 1999. MRID 49009507. Unpublished. 35 pages.

In a preliminary range-finding feeding study (MRID 49009507), paraquat dichloride technical (32.32% a.i.; Batch no. D9485/072) was administered in feed to one group of six female New Zealand White rabbits in ascending dietary concentrations for four to seven days at each concentration with 2- or 3-day untreated "rest" periods in between the treatment intervals. The succession of dietary concentrations was as follows: 42 ppm (for 4 days), 80 ppm (for 4 days), 179 ppm (for 5 days), and 332 ppm (for 7 days) intended to result in dose levels of 2, 4, 8, or 12 mg/kg bw/day, respectively. [Note: there was a 2-day rest period between the 179- and 332-ppm treatment intervals, and the remaining rest periods were 3 days.] Evaluated parameters included clinical signs, body weight, food consumption, and compound consumption, with scheduled sacrifice and gross necropsies done on day 29.

The mean (±SD) doses received by the rabbits fed at the 42-, 80-, 179-, and 332-ppm dietary concentrations were as follows: 2.1±0.3 mg/kg bw/day (with individual daily values ranging 1.6-2.9 mg/kg bw/day); 3.6±0.5 mg/kg bw/day (individual daily values: 2.9-5.0 mg/kg bw/day); 6.6±1.4 mg/kg bw/day (individual daily values: 3.5-10.1 mg/kg bw/day); and 9.1±3.1 mg/kg bw/day (individual daily values: 3.6-17.6 mg/kg bw/day).

There were no deaths or abnormal clinical signs. Treatment at 42 ppm did not affect body weight gain or food consumption. Relative to food consumption during acclimation, there were dose-related decreases in food consumption during the 80-, 179-, and 332-ppm treatment intervals with compensatory increases seen during the rest periods. After three days at the 332-ppm treatment level, the amount of food consumed gradually increased during the remaining four days of this period but remained significantly lower than that seen prior to treatment. Body weight gain was decreased at 80 ppm, and body weight losses were seen at 179 and 332 ppm. Gross necropsy findings were limited to prominent Peyer's patches in the jejunum of one animal and prominent Peyer's patches in the ileum of a different animal. The study author noted, and the reviewer concurs, that it is unknown whether the effects on food consumption were a direct treatment-related effect or secondary to altered palatability of the diet resulting from inclusion of the test material. The results also highlight the difficulty of maintaining a steady dose level in terms of mg/kg bw/day through the inclusion of paraquat dichloride in the diet at a fixed concentration.

Under the conditions of this study, the lowest-observed-effect level (LOEL) was 80 ppm (3.6 mg/kg bw/day) based on decreased body weight gain and food consumption, and the NOEL was 40 ppm (2.1 mg/kg bw/day).

Based on these results, targeted dose levels of 8 and 12 mg/kg bw/day were selected for a 13-day study using fixed dietary concentrations of paraquat dichloride.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. According to the GLP Compliance Statement, the studies were not conducted in compliance with OECD Principles of Good Laboratory Practice (1997). A Quality Assurance statement was not provided. A Statement of Authentication signed by the study director (p. 6) included the following assertion: The data described in this report have not been subjected to audit by the Laboratory's Quality Assurance Unit but are derived from a study which is considered to meet the principles of Good Laboratory Practice.

DATA EVALUATION RECORD

PARAQUAT DICHLORIDE

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY – RABBIT
OCSPP 870.3700b [§83 3b]; OECD 414

MRIDS 49009505 (Main Study) and 49009502, 49009503, 49009504

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Work Assignment Manager: Lori Brunsman **Signature:**
Science Info. Mgmt. Branch, Health Effects Division (7509P) **Date:** 7/17/14

TXR#: 0056764

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OCSPP 870.3700b [§83-3b]; OECD 414.

PC CODE: 061601 **DP BARCODES:** D409213

TEST MATERIAL (PURITY): Paraquat (33.6% w/w paraquat ion)

SYNONYMS: Gramoxone YF6219

CITATIONS: Tinston, D. (1991) Paraquat - second teratogenicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2763, September 26, 1991. MRID 49009505. Unpublished.

Hodge, M. (1990) Paraquat - embryotoxicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/L/3423, October 18, 1990. MRID 49009502. Unpublished.

Tinston, D. and J. Barber (1991) Paraquat - second embryotoxicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2730, July 11, 1991. MRID 49009503. Unpublished.

Tinston, D. (1991) Paraquat - teratogenicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2749, September 26, 1991. MRID 49009504. Unpublished.

SPONSOR: ICI Agrochemicals. The submitter is Syngenta Crop Protection, LLC, 410 Swing Road, Greensboro, North Carolina.

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 49009505), paraquat (33.6% w/w paraquat ion; Lot/batch #YF6219 [ex No. 9 Product Stock Tank]) was administered to twenty inseminated female New Zealand White rabbits/dose by gavage in deionized water at dose levels of 0, 1.0, 1.5, or 2.0 mg/kg bw/day (dose volume 1 mL/kg bw) on gestation days (GDs) 7 through 19, inclusive, with the day of insemination designated as GD 1. On GD 30, surviving does were

sacrificed and necropsied. Gravid uterine weights, corpora lutea counts, and the numbers and positions of live fetuses and early and late intrauterine deaths were recorded. Fetuses were weighed, examined for external anomalies (including cleft palate), sexed internally, subjected to visceral examination, and fixed in methanol. Following 24 hours fixation, the head of each fetus was cut along the fronto-parietal suture line, and the brain was examined, with the carcass subsequently processed for and subjected to skeletal examination. This study repeated a previous study (MRID 49009504) for which dose selection was based on two preliminary studies (MRIDs 49009502 and 49009503). [See Appendices I, II, and III of this DER for summaries.]

Mortality occurred in all treated groups, with 20, 17, 12, and 9 animals surviving to scheduled termination in the 0-, 1.0, 1.5, and 2.0 mg/kg groups, respectively. Two 2.0 mg/kg animals were found dead (GDs 21 and 22), and two, four, and four animals from the 1.0 , 1.5 , and 2.0 mg/kg groups, respectively, were killed between GDs 12 and 24 because of excessive weight loss and/or poor clinical condition. In addition, one, four, and five animals in the 1.0, 1.5, and 2.0 mg/kg groups, respectively, were killed following abortions (GDs 28, 20-29, and 21-30, respectively). There were no mortalities or abortions in the control group. Treatment-related clinical signs included few or no feces, thin appearance, and abnormal feces (diarrhea, signs of diarrhea, or mucus or blood). Significant ($p<0.05$ or 0.01) dose- and treatment-related mean body weight losses were seen at all dose levels during GD 7-19 (BW changes: +201.4 g, -98.0 g, -194.3 g, and -363.8 g in ascending dose order). There were correlated statistically significant treatment-related decreases in food consumption in 1.0 mg/kg animals during GD 10-16 (33-34% less than controls) and in 1.5 and 2.0 mg/kg animals throughout treatment (38-49% and 46-67% less than controls, respectively). These effects were most pronounced in animals that aborted, had total litter resorptions, or died/were sacrificed intercurrently. Potentially treatment-related gross findings were noted among the animals that were found dead or sacrificed ahead of schedule. These included stomach lesions such as ulceration of the glandular or non-glandular portion, pale or hemorrhagic areas, distension, abnormal contents, and/or thin wall (in 2/3, 4/8, and 7/11 animals) and fluid colon contents (in 1/3, 2/8, and 4/11 animals). **Under the conditions of this study, the maternal lowest-observed-adverse-effect level (LOAEL) for paraquat dichloride in rabbits dosed on days 7-19 of gestation (with day of insemination = GD 1) is 1.0 mg/kg bw/day, based on death, abortion, weight loss, decreased food consumption, and gross pathology of the stomach and colon. The maternal NOAEL is not identified.**

Relative to controls, all treated groups had lower mean numbers of corpora lutea (11.47, 10.18, 10.44, and 9.63 for 0-, 1.0, 1.5, and 2.0 mg/kg groups, respectively). This, in conjunction with higher mean percentages preimplantation loss (8.3%, 15.3%, 20.9%, and 18.3%) resulted in lower mean numbers of implantations (10.53, 8.73, 8.56, 7.88; $p<0.05$) and lower mean numbers of live fetuses (9.59, 8.18, 6.00, and 7.38; $p<0.05$ at 1.5 and 2.0 mg/kg bw/day). The higher percentages or preimplantation loss in the treated groups and associated decreases in mean numbers of implantations and live fetuses may be treatment-related, reflecting very early resorptions (i.e. on GDs 7-8); however, it is also possible that the differences are related to animal husbandry issue(s), insemination technique, or compromised animal health. The 1.5 mg/kg group had an increased mean percentage postimplantation loss (20.9% vs. 8.1% for controls; $p<0.01$) that was associated with an increased proportion of dams with at least one early intrauterine death (55.6% vs. 5.9% of controls). Two total litter resorptions (one in each of the 1.0 and 1.5 mg/kg groups) were considered treatment-related. Mean fetal weight and fetal sex ratio were not affected by treatment.

The total numbers of fetuses (and litters) evaluated in the control, 1.0, 1.5, and 2.0 mg/kg groups were 163 (17), 90 (11), 54 (9), and 59 (8), respectively. In these same respective groups, major defects were observed in a total of 3 (3), 3 (3), 3 (3), and 2 (1) fetuses (and litters). These included major head defects in 1 (1) and 2 (1) 1.5 and 2.0 mg/kg fetuses (and litters), respectively, limb defects in 1 (1) and 3 (3) 1.0 and 1.5 mg/kg fetuses (and litters), respectively, and spina bifida occulta in one 1.0 mg/kg fetus. At 2.0 mg/kg bw/day there were significant ($p<0.05$) treatment-related increases in the litter incidences of "27 pre-sacral vertebrae" and "27 pre-sacral vertebrae with any extra 13th rib" (100% vs. 52.9% of control litters for both), and the incidence of "any extra 13th rib" was significantly increased at all treatment levels (50.9%, 68.9%, 72.2%, and 79.7% of fetuses with >88% of litters affected in all groups). Although treatment-related, the increased incidences of variants are not considered adverse. **The developmental LOAEL for paraquat dichloride in rabbits dosed on days 7-19 of gestation (with day of insemination = GD 1) is 1.0 mg/kg bw/day, based on abortion and total litter resorption. The developmental NOAEL is not identified.**

In light of the fact that the disparity between the results of this study and MRID 49009504 calls into question the conduct of the study, and because the study was not conducted in compliance with the Principles of Good Laboratory Practice, no Quality Assurance statement was provided, and no individual data were provided, this developmental toxicity study in the rabbit is classified **Unacceptable/Guideline** and *does not satisfy* the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) in the rabbit.

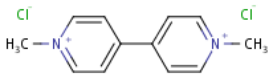
COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. According to the GLP Compliance Statements, the studies were not conducted in compliance with OECD Principles of Good Laboratory Practice (1997). Quality Assurance statements were not provided, and the original title pages stated, "The data in this report have not been quality assured."

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Paraquat

Description:	Dark brown/black liquid
Batch #:	YF6219 (ex No. 9 Product Stock Tank); CTL reference no. Y00061/160
Purity:	33.6% w/w paraquat ion
Compound stability:	Not reported
CAS # of TGAI:	1910-42-5
Structure:	



2. Vehicle and/or positive control: The vehicle was deionized water. A positive control was not used.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Age/weight at study initiation:	Males: age/weight not reported Females: age not reported/3.3-4.6 kg on the day of insemination
Source:	Interfauna UK (Huntingdon, Cambridgeshire, U.K.)
Housing:	Individually in suspended cages in "mobile rabbit units"
Diet:	CRB pellets (Labsure Animal Diets, Manea, Cambridgeshire, U.K.), *ad libitum*
Water:	Filter-sterilized municipal tap water, via automatic watering system, *ad libitum*
Environmental conditions:	**Temperature:** 15-19°C **Humidity:** 40-70% **Air changes:** 25-30/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	At least two weeks.

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: March 4, 1991; End: April 12, 1991.

2. Mating: Approximately two weeks prior to shipment, the supplier gave each female an intravenous injection of 25 IU chorionic gonadotrophin [PROFASI 500, Serono Laboratories (UK) Ltd] in order to promote ovulation prior to insemination. Semen was collected from proven males of the same source and strain as the females, using an artificial vagina. Each collected sample was diluted with enough Medium 199 culture media (Flow Laboratories Limited, Irvine, Ayrshire, Scotland) to attain sufficient volume to inseminate four females

(one replicate). Approximately 1 mL of the diluted semen was inseminated into each female using a plastic catheter (12F6 Nelaton supplied by Portex Limited, Hythe, Kent, UK). Each replicate of females was inseminated with semen from one male, and a total of eleven males were used. After insemination, each female was given an intravenous injection of 25 IU human chorionic gonadotrophin (PROFASI 500) to promote ovulation. The day of insemination was designated as gestation day (GD) 1. It must be noted that there was no mention of evaluation of the semen for sperm count, morphology, or motility, and the exact timing of the HCG injections was not reported.

3. **Animal Assignment:** Animal assignment is given in Table 1. The study was divided into twenty replicates (randomized blocks), each of which contained one inseminated female from each group. Cages within the replicates were assigned to one of the four groups using computer-generated random number permutations. Following insemination, each female was randomly allocated to a cage (and therefore a treatment group) within the replicate. Replicates were filled sequentially.

TABLE 1. Animal assignment [a]

Group	Control	Low-Dose	Mid-Dose	High-Dose
Dose (mg/kg bw/day)	0	1.0	1.5	2.0
Number of Females	20	20	20	20

[a] Data taken from text table, p. 16, MRID 49009508.

4. **Dose selection rationale:** The dose levels were selected to coincide with those used in a previously conducted developmental toxicity study with the same test material in rabbits (MRID 49009504, summarized in Appendix III of this DER), in which the pregnancy rates were too low to fulfill the objectives of the study. Dose selection for MRID 49009504 was based on the results from two preliminary developmental toxicity studies in the rabbit (MRIDs 49009502 and 49009503, summarized in Appendices I and II, respectively, of this DER).

5. **Dosage preparation and analysis:** Test material-vehicle mixtures were prepared on two occasions (March 7 and 18, 1991) by adding appropriate amounts of deionized water to weighed quantities of the test substance (adjusted for purity) and then shaking by hand until solutions were formed. The preparations (including vehicle control) were subdivided into aliquots and stored at room temperature until used. Stability of the test substance in deionized water over a period of 28 days storage at room temperature was evaluated in conjunction with the previously conducted developmental toxicity study (MRID 49009504). Homogeneity of the test mixtures was not evaluated.

Results:

Stability analysis: The mean measured concentrations of the low-dose (1.0 mg/mL) and high-dose (2.0 mg/mL) mixtures were both 98.0% of their initial concentrations after 28 days of storage at room temperature.

Concentration analysis: The mean measured concentrations of the low-, mid-, and high-dose mixtures were 96.0-97.0%, 95.3-97.3%, and 92.5 98.0% of nominal, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses were administered once daily by gavage, on GDs 7 through 19, in a volume of 1 mL/kg of body weight. Dosing was based on the most recent daily body weight determination.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for mortality or clinical signs at least twice daily. Body weights were recorded on GDs 1 and 4, 7-19, and 22, 26, and 30. Food consumption was measured over the following intervals: GDs 1-4, 4-7, 7-10, 10-13, 13-16, 16-19, 19-22, 22-26, and 26-30. On GD 30, surviving females were euthanized by intravenous sodium pentobarbital (Euthatal) and subjected to a gross necropsy. Gravid uterine weights, corpora lutea counts, and the numbers and positions of implantations (live fetuses and early and late intrauterine deaths) were recorded. Intrauterine deaths were categorized as early if they consisted of decidual or placental tissue only and were categorized as late if embryonic or fetal tissue was present in addition to placental tissue. The study author did not mention usage of ammonium sulfide or sodium hydroxide staining technique to detect very early resorptions. Animals that died or were sacrificed due to moribundity or abortion were subjected to gross necropsy, including evaluation of pregnancy status. Data were tabulated in summary form only; individual data were not provided.

2. **Fetal evaluations:** All fetuses were weighed, tagged, and euthanized via intrathoracic injection of sodium pentobarbital. Following external examination, including checking for cleft palate, fetuses were sexed internally, subjected to visceral examination, eviscerated, and fixed in methanol. Following 24 hours fixation, the head of each fetus was cut along the fronto-parietal suture line, and the brain was examined. Carcasses were further processed and stained with Alizarin Red S for skeletal examination.

   External, visceral, and skeletal findings were classified as major defects, minor defects, or variants. See Appendix IV of this DER for the description of the categories.

   The individual bones of the *manus* and *pes* were assessed, and the results were separately converted to a six-point ossification scale, in which a score of 1 was "good," and a score of 6 was "poor." See Appendix V of this DER for the details of the scaling method.

   Data were tabulated in summary form only; no individual data were provided.

D. DATA ANALYSIS:

1. **Statistical analyses:** Data were analyzed as follows.

   Analysis of variance (ANOVA) was used to evaluate the following: maternal bodyweight gain, maternal food consumption, mean numbers of implantations and live fetuses per dam, percentages pre- and postimplantation loss, percentage of male fetuses, gravid uterine weight, litter weight, and fetal weight, mean *manus* and *pes* score, percentage of fetuses with minor

external/visceral defects only and minor skeletal defects only. Prior to analysis, percentages were transformed using the double arcsine transformation of Freeman and Tukey (1950), and fetal data were converted to the litter level.

Fisher's Exact Test was used to evaluate the proportions of females with pre- or postimplantation losses or early or late intrauterine deaths, the proportion of male fetuses, the proportion of fetuses with major or minor (only) external/visceral defects, major or minor (only) skeletal defects, skeletal variants, and each specific finding. The proportion of fetuses with each specific finding was also analyzed on a litter basis.

All tests used a minimum significance level of $p<0.05$, and one-sided statistical tests were used, except for maternal bodyweight gain, maternal food consumption, and the proportion of fetuses that were male. Analyses were done both including and excluding animals that died intercurrently, aborted, or had total litter resorptions.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

$$\text{Preimplantation Loss}(\%) = \left[\frac{\text{No. of Corpora Lutea} - \text{No. of Implantations}}{\text{No. of Corpora Lutea}}\right] \times 100$$

$$\text{Postimplantation Loss}(\%) = \left[\frac{\text{No. of Implantations - No. of Live Fetuses}}{\text{No. of Implantations}}\right] \times 100$$

3. **Historical control data:** Historical control data were not provided.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** Selected clinical signs data are given in Table 2, and data pertaining to mortality and abortions are presented in Table 5.

Two 2.0 mg/kg animals were found dead (GDs 21 and 22), and two 1.0 mg/kg animals, four 1.5 mg/kg animals, and four 2.0 mg/kg animals were killed following excessive weight loss and/or poor clinical condition (GDs 21-24, 12-24, and 22-23, respectively). In addition, one, four, and five animals in the 1.0, 1.5, and 2.0 mg/kg groups, respectively, were killed following abortions (GDs 28, 20-29, and 21-30, respectively). There were no mortalities in the control group.

Treatment-related clinical signs included few or no feces, thin appearance, abnormal feces (diarrhea, signs of diarrhea, or mucus or blood). These signs were seen at increased incidences or in increased numbers of animals at all treatment levels, relative to controls. Vaginal bleeding was noted from two 1.0 mg/kg animals (one each on GDs 19 and 21), and blood on the tray was recorded for treated animals but not for controls; however, not all of the observations of blood on the tray were treatment-related, as the earliest observation of this sign at the 1.5 mg/kg dose level was on GD 3, prior to the initiation of treatment. Other

findings were either of a low incidence and/or showed no dose-response and were considered to be incidental to treatment.

TABLE 2. Selected clinical observations (total occurrence/ # of animals [time of occurrence]) [a]

Observation	Dose in mg/kg bw/day			
	Control	1.0	1.5	2.0
Thin	7/1 [GD 1-7]	26/4 [GD 19-30]	29/7 [GD 11-29]	20/6 [GD 17-29]
Few feces on tray	18/9 [GD 2-27]	45/11 [GD 3-30]	102/17 [GD 3-30]	99/18 [GD 8-30]
No feces on tray	3/2 [GD 1-25]	58/7 [GD 4-30]	86/15 [GD 7-30]	96/13 [GD 8-29]
Diarrhea	2/1 [GD 20-21]	6/1 [GD 1-22]	13/4 [GD 1-22]	0
Signs of diarrhea	0	0	7/4 [GD 6-30]	7/4 [GD 12-24]
Mucus in feces	0	1/1 [GD 19]	2/2 [GD 8 and 19]	4/1 [GD 18-21]
Blood in feces	0	0	2/1 [GD 7-8]	0
Blood on tray	0	9/3 [GD 19-23]	7/4 [GD 3-30]	9/2 [GD 20-29]
Blood in urine	1/1 [GD 26]	0	0	0
Vaginal bleeding	0	2/2 [GD 19 and 21]	0	0
Abnormal respiratory noise	0	11/1 [GD 2-12]	0	0

[a] Data taken from Table 5, pp. 38-41, MRID 49009505.

2. **Body weight:** Selected body weight data are given in Table 3. Marked dose- and treatment-related mean body weight losses were seen at all dose levels during dosing. Although the losses were most pronounced in the animals that aborted, had total litter resorptions, or died/were sacrificed intercurrently, body weight gain was also adversely affected in the animals that survived to terminal sacrifice. For pregnant survivors, all groups had non-statistically-significant decreases in body weight gain over the GDs 7-19 treatment interval relative to controls (-29%, -60%, and -63% for 1.0 , 1.5, and 2.0 mg/kg groups, respectively; data not shown) with transient mean weight loss in 1.5 and 2.0 mg/kg animals during days 7-10 (-69.9 and -50.3 g, respectively, vs. +17.2 g for controls; data not shown). Surviving animals with at least one live fetus at necropsy did not exhibit clear effects on cumulative body weight gain or the adjusted (for gravid uterine weight) cumulative body weight gain during GDs 1-30.

TABLE 3. Mean maternal body weight data (g) [a]

Gestation Day or Interval	Dose in mg/kg bw/day [# of Dams]			
	Control [n=17]	1.0 [n=11-15]	1.5 [n=9-16]	2.0 [n=8-19]
Absolute weight				
GD 1 (Initial body weight)	4030.8	4015.5	4079.4	4012.1
Gravid uterus	621.5	530.2 * (-15) [b]	451.8 ** (-27)	489.5 * (-21)
Body weight changes				
Pre-dosing: GD 1-7	128.3	139.4	93.0	164.5
Dosing: GD 7-10	17.2	-27.2	-78.1 **	-132.5 **
GD 10-13	60.2	-29.5	-63.0 **	-107.9 **
GD 13-16	102.7	-0.1	1.3	-44.6 **
GD 16-19	21.3	-41.2	-75.6 **	-78.8 **
GD 7-19	201.4	-98.0 *	-194.3 **	-363.8 **
Post-dosing: GD 19-30	221.7	191.4	139.1	185.0
Cumulative: GD 1-30 [c]	551.4	548.9	389.1	552.0
Net change: GD 1-30 [c d]	-70.1	18.7	-62.7	62.5

a Data taken and/or derived from Table 6.3, pp. 45 MRID 49009505. Note: standard deviations were not provided in the study report. Data from animals the died or were sacrificed intercurrently are included, except where indicated.

b Numbers in parentheses equal percent different from control; calculated by reviewer.

c Data excluded from dams that aborted or had total litter resorptions.

d Calculated by reviewer, using group mean values, as net weight change = cumulative BW change - gravid uterine weight; not subjected to statistical analysis.

* Significantly different ($p<0.05$) from controls.

** Significantly different ($p<0.01$) from controls.

3. <u>Food consumption</u>: Selected food consumption data are given in Table 4. The statistically significant decreases in food consumption in 1.0 mg/kg animals during GD 10-16 and in 1.5 and 2.0 mg/kg animals throughout treatment were considered treatment-related and adverse.

TABLE 4. Mean maternal food consumption data (g/animal/day) [a]					
Gestation Day or Interval		Dose in mg/kg bw/day [# of Dams]			
		Control [n=17]	1.0 [n=12-15]	1.5 [n=10-16]	2.0 [n=9-19]
Pre-dosing:	**GD 1-7**	191.6	194.4	183.1	195.9
Dosing:	**GD 7-10**	175.5	156.9	90.0 ** (-49) [b]	75.1 ** (-57)
	GD 10-13	190.2	127.7 * (-33)	96.3 ** (-49)	63.7 ** (-67)
	GD 13-16	175.7	115.5 * (-34)	99.8 ** (-43)	67.5 ** (-62)
	GD 16-19	162.7	126.9	100.1 * (-38)	87.8 * (-46)
	GD 7-19	178.4	138.1	99.5 ** (-44)	76.3 ** (-57)
Post-dosing:	**GD 19-30**	148.4	159.8	154.3	170.4

[a] Data taken from Table 7.3, p. 48, respectively, MRID 49009505. Note: standard deviations were not provided in the study report.

[b] Numbers in parentheses equal percent different from control; calculated by reviewer.

* Significantly different ($p<0.05$) from controls.

** Significantly different ($p<0.01$) from controls.

4. **Gross pathology:** A number of gross findings were noted among the three 1.0 mg/kg, eight 1.5 mg/kg, and eleven 2.0 mg/kg females that were found dead or sacrificed ahead of schedule. Stomach lesions such as ulceration of the glandular or non-glandular portion, pale areas, distension, abnormal contents, sloughing of the mucosa when washed, hemorrhagic areas, and/or thin wall were noted in 2/3, 4/8, and 7/11 animals. Fluid colon contents were noted in 1/3, 2/8, and 4/11 animals. Distended gallbladder was noted in 3/3, 3/8, and 3/11 animals, but this finding is common in all species after a prolonged period of fasting and is not considered by the reviewer to be adverse. The remaining findings were seen in just one to two animals and without a clear dose response, and therefore were not considered treatment-related.

   None of the gross findings among survivors were considered treatment-related due to being present at low incidences (i.e., one or two animals) or without evidence of a dose response. The gastric mucosa sloughed off when washed in 3/20, 1/17, 0/12, and 1/9 surviving 0-, 1.0 , 1.5 , and 2.0 mg/kg animals, respectively.

5. **Cesarean section data:** Data collected at cesarean section are given in Table 5. Relative to controls, all treated groups had lower mean numbers of corpora lutea, higher mean percentages preimplantation loss, and lower mean numbers of implantations and live fetuses. The decreased live litter sizes correlated with decreased mean gravid uterus weights and litter weights, while mean fetal weights (for the sexes combined) of the treated groups remained similar to the control value. An increased mean percentage postimplantation loss for the 1.5 mg/kg group was largely, but not entirely, due to one dam (#47) that had one early and eleven late intrauterine deaths out of thirteen implantations; however, this group also had an increased proportion of dams that had at least one early intrauterine death. There were two total litter resorptions: one in each of the 1.0 and 1.5 mg/kg groups; no information was available concerning the numbers of total or early/late intrauterine deaths in these dams. While differences in mean numbers of corpora lutea cannot be attributed to treatment, the higher percentages preimplantation loss in the treated groups (and associated decreases in

mean numbers of implantations and live fetuses) may reflect resorptions that occurred too early in gestation to leave an implantation scar (i.e. on GDs 7-8, when the day of insemination is designated as GD 1, as it was in this study). Moreover, it is unknown whether ammonium sulfide or sodium hydroxide staining technique to detect very early resorptions was done. The increased preimplantation loss in all treated groups and the increased postimplantation loss in the 1.5 mg/kg group also may both be treatment-related in this study, despite the absence of a clear dose-response for both of these differences. The higher numbers of treatment-related abortions in the 2.0 mg/kg group may have been related to intrauterine deaths/postimplantation losses.

TABLE 5. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	1.0	1.5	2.0
# Animals assigned (inseminated)	20	20	20	20
# Animals pregnant	17	15	16	19
% animals pregnant	85%	75%	80%	95%
Maternal wastage [b]	0	3	8	11
# Died	0	0	0	2
# Sacrificed *in extremis* or for humane reasons	0	2	4	4
# Aborted/delivered early	0	1	4	5
# Animals pregnant at necropsy	17	12	10	8
# Animals with total litter resorptions	0	1	1	0
Total # of litters (with live fetuses) evaluated at c-section	17	11	9	8
Mean # corpora lutea per dam	11.47	10.18	10.44	9.63
Mean # implantations per dam	10.53	8.73 *	8.56 *	7.88 *
Mean # of live fetuses per dam	9.59	8.18	6.00 **	7.38 *
Total # of intrauterine deaths	16	6	23 [11] [c]	4
Early	4	1	9 [8]	2
Late	12	5	14 [3]	2
Mean intrauterine deaths/dam [d]	0.94	0.53	2.56 [1.38]	0.50
Early [d]	0.24	0.09	1.00 [1.00]	0.25
Late [d]	0.71	0.45	1.56 [0.38]	0.25
# Dams with at least 1 intrauterine death	8	5	6 [5]	4
Percentage [d]	47.1%	45.5%	66.7% [62.5%]	50.0%
Mean intrauterine deaths/affected dam [d]	2.00	1.20	3.83 [2.20]	1.00
# Dams with at least 1 early intrauterine death	1	1	5 [4]	2
Percentage [d]	5.9%	9.1%	55.6% ** [50.0%]	25.0%
Mean early intrauterine deaths/affected dam [d]	4.00	1.00	1.80 [2.00]	2.00
Mean litter weight	399.8	344.9 (-14) [e]	265 ** (-34)	311.7 * (-22)
Mean fetal weight (g)	42.39	43.58	42.41	42.61
Sex ratio (mean % male)	53.2	39.9	42.5	43.7
Preimplantation loss (mean %)	8.3	15.3	20.9 *	18.3
Postimplantation loss (mean %)	8.1	9.2	22.9 *	6.6

[a] Data taken from Tables 4 and 9, pp. 36 and 53-55, respectively, MRID 49009505.

[b] Includes *all* deaths and animals sacrificed *in extremis* or for humane reasons; pregnancy status of these animals was not provided.

[c] Numbers in brackets are excluding data from the animal that had 1 early and 11 late intrauterine deaths and just 1 live fetus; calculated by reviewer.

[d] Calculated by reviewer.

[e] Numbers in parentheses equal percent different from control; calculated by reviewer.

* Significantly different ($p<0.05$) from controls.

** Significantly different ($p<0.01$) from controls.

B. <u>DEVELOPMENTAL TOXICITY</u>: The total numbers of fetuses (and litters) evaluated in the control, 1.0, 1.5, and 2.0 mg/kg groups were 163 (17), 90 (11), 54 (9), and 59 (8), respectively. In these same respective groups, major defects were observed in a total of 3 (3), 3 (3), 3 (3), and 2 (1) fetuses (and litters). Major defects are given in Table 6a; minor external and visceral defects are given in Table 6b; and selected minor skeletal defects and

skeletal variants are given in Table 6c. Note: the study author reported and discussed the findings from the external and visceral exams together, as a single category, in the study report; for clarity, the reviewer has done the same thing in this DER.

1. **External and visceral examinations:** In the control, 1.0, 1.5, and 2.0 mg/kg groups, the total numbers of fetuses (and litters) with major visceral/external defects were 2 (2), 3 (3), 3 (3), and 2 (1), respectively, (mean percentages per litter: 1.2%, 2.8%, 14.6%, and 2.5%). In these same respective groups, the mean percentages per litter with *only* minor defects were 9.3%, 5.8%, 2.3%, and 10.0%. No external/visceral variants were seen.

   The mean percentage of fetuses per litter with major defects was increased in the 1.5 mg/kg group due to one female (#47) whose only live fetus had limb defects. There were no statistically significant increases in the litter incidences of the specific visceral/external findings or in the overall litter incidences of major or minor visceral/external defects. Of note, however, were the occurrences of major head defects and major limb defects in treated groups but not in controls.

2. **Skeletal examination:** In the control, 1.0, 1.5, and 2.0 mg/kg groups, the total numbers of fetuses (and litters) with major skeletal defects were 1 (1), 2 (2), 1 (1), and 2 (1), respectively, (mean percentages per litter: 0.6%, 1.8%, 2.2%, and 2.5%). In these same respective groups, the mean percentages per litter with *only* minor defects were 44.8%, 58.2%, 42.0%, and 45.2%, and the mean percentages per litter with variants were 91.8%, 99.1%, 95.0%, and 98.8%.

   The major defects noted in the 1.5 and 2.0 mg/kg groups were skull findings in the same fetuses that had head defects on external and/or visceral examination. Spina bifida occulta (involving widespread arches from the 6th lumbar to the 4th sacral vertebrae) was noted in one of the affected 1.0 mg/kg fetuses and is of interest due to being another neural tube defect.

   There were treatment-related increased incidences of several skeletal variants and "syndromes" of variants. These included 27 pre-sacral vertebrae, 27 pre-sacral vertebrae with any extra 13th rib (both in the 2.0 mg/kg group), and any extra 13th rib (in all treated groups). There were no statistically significant differences in the litter incidences of the specific minor skeletal defects or in the overall litter incidences of minor skeletal defects. However, in the 2.0 mg/kg group, there were slight, non-statistically significant increases in the litter and fetal incidences of asymmetrical alignment of the pelvic girdle, a minor defect. The study author stated that "the occurrence of 27 pre-sacral vertebrae and also asymmetrical alignment of the pelvic girdle was associated with the presence of extra thoracic ribs, and a dose-related trend was evident for the paraquat treated groups although the percentage of fetuses affected in the l.0-mg/kg/day group was not statistically significantly different from the control group." It is unclear to the reviewer whether the study author meant that these three findings tended to co-occur in the same fetuses. The data provided in the summary table included the litter and fetal incidences of the syndrome of 27 pre-sacral vertebrae with any extra 13th rib but did not include data pertaining to the observation of asymmetrical alignment of the pelvic girdle in fetuses that had the other two findings, and, as previously mentioned, the individual data were not provided.

The two most common minor skeletal defects were partially ossified 6th sternebra and unossified transverse processes of the 7th lumbar vertebra, which were seen at similar litter and fetal incidences in all groups. The most common variants were partially ossified odontoid and partially ossified 5th sternebra, both of which were seen in ≥75% of the litters and ≥35% of the fetuses in all groups. Statistically significant increases in the fetal incidences of partially ossified 5th sternebra in the 1.0 and 2.0 mg/kg groups (but not the 1.5 mg/kg group) were correlated with non-statistically significant decreases in the fetal incidences of unossified 5th sternebra (a minor defect) in the same two groups; these differences were considered non-treatment-related in light of the absence of a dose response or any other indications of altered ossification or growth.

For the 0-, 1.0, 1.5, and 2.0 mg/kg groups, the mean *manus* scores were 3.05, 2.91, 2.92, and 3.05, respectively, and the mean *pes* scores were 1.14, 1.00, 1.14, and 1.10 for these same respective groups.

TABLE 6a. Major defects [a] [Fetal (litter) incidences]

Observations [b]	Dose (mg/kg bw/day)			
	0	**1.0**	**1.5**	**2.0**
Number examined	163 (17)	90 (11)	54 (9)	59 (8)
Total affected	3 (3)	3 (3)	3 (3)	2 (1)
Oral atresia, nares absent, cyclopia, external hydrocephaly, gross malformation of the skull, major cervical defect	0	0	0	1 (1)
Internal hydrocephaly, cebocephaly, maxilla fused	0	0	0	1 (1)
Acephaly (agenesis of the skull)	0	0	1 [c] (1)	0
Anomaly of the great vessels of the heart	0	0	1 [c] (1)	0
Aorta enlarged, pulmonary artery reduced	1 [d] (1)	2 [e] (2)	0	0
Aorta reduced, pulmonary artery enlarged	1 (1)	0	0	0
Persistent ductus arteriosis, single ventricle heart	1 [d] (1)	0	0	0
Right hindlimb malrotated, left hindlimb extremely flexed	0	0	1 [g] (1)	0
Forepaw extremely flexed	0	0	2 [c] (2)	0
Hindlimb extremely flexed	0	1 [f] (1)	0	0
Spina bifida occulta, 6th lumbar to 4th sacral arches wide spread	0	1 [f] (1)	0	0
Extra vertebral arch between the 7th cervical and 1st thoracic	0	1 [e] (1)	0	0
3rd lumbar arch not ossified	1 (1)	0	0	0

a Data taken from Tables 10, 11, and 12, pp. 56-57, 58-59, and 61-72, respectively, MRID 49009505.

b Some observations may be grouped together.

c, d, e, f Malformations observed together in one fetus.

g Fetus weighed 19.2 g and was the only survivor in a litter with one early and eleven late intrauterine deaths.

TABLE 6b. External and visceral minor defects [a] [Fetal (litter) incidences]				
Observations [b]	Dose (mg/kg bw/day)			
	0	1.0	1.5	2.0
Number examined	163 (17)	90 (11)	54 (9)	59 (8)
Heart: thickened ventricle wall	1 (1)	0	0	0
Extra blood vessel between left carotid and left subclavian	1 (1)	0	1 (1)	0
Liver: Cyst(s) attached	11 (9)	4 (1)	1 (1)	6 (4)
Mottled	1 (1)	0	1 (1)	0
Gallbladder: Bilobed	1 (1)	0	0	0
Spleen: Secondary spleen	0	1 (1)	0	0
Ureter: Kinked	1 (1)	0	0	0
Forepaw: Slightly flexed	2 (2)	0	1 (1)	0

[a] Data taken from Tables 10 and 12, pp. 56-57 and 61-72, respectively, MRID 49009505.

[b] Some observations may be grouped together.

TABLE 6c. Selected skeletal minor defects and variants [a] [Fetal (litter) incidences]				
Observations [b]	Dose (mg/kg bw/day)			
	0	1.0	1.5	2.0
Number examined	163 (17)	90 (11)	54 (9)	59 (8)
Minor defects				
Sternebrae: 5^{th} not ossified	19 (10)	8 (4)	9 (6)	3 (3)
6^{th} partially ossified	25 (12)	21 (10)	4 (3)	6 (4)
Transverse processes of 7^{th} lumbar vertebra not ossified	19 (8)	10 (5)	3 (3)	3 (2)
Hyoid misshapen	3 (3)	5 (3)	4 (3)	3 (2)
Pelvic girdle: asymmetric alignment	5 (4)	3 (2)	1 (1)	5 (3)
Variants				
27 Pre-sacral vertebrae	26 (9)	20 (9)	21 ** (6)	31 ** (8 *)
Any extra 13^{th} rib	83 (15)	62 ** (10)	39 ** (9)	47 ** (8)
Extra 13^{th} rib - normal length	57 (14)	32 (10)	28 * (7)	36 ** (8)
Extra 13^{th} rib - normal length and floating	3 (2)	4 (2)	0	4 (4)
Extra 13^{th} rib - short length	35 (13)	22 (8)	14 (6)	11 (6)
Extra 13^{th} rib - short length and floating	16 (10)	16 (7)	4 (3)	5 (3)
27 Pre-sacral vertebrae with any extra 13^{th} rib	25 (9)	20 (9)	19 ** (5)	31 ** (8 *)
Sternebrae: 5^{th} partially ossified	74 (16)	57 ** (10)	24 (7)	35 ** (8)
Odontoid partially ossified	69 (16)	38 (10)	23 (7)	21 (6)

[a] Data taken from Tables 10 and 12, pp. 56-57 and 61-72, respectively, MRID 49009505.

[b] Some observations may be grouped together.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

There was no evidence of disease or infection in the animals which might have compromised the study.

Overt maternal toxicity was evident in all treated groups as a dose-related increase in the incidence of animals found dead or killed due to excessive weight loss or deterioration in clinical condition. Marked reductions in bodyweight gain and food consumption were seen in the 1.5 and 2.0 mg/kg groups and in the 1.0 mg/kg group when all animals were included. Associated clinical findings were reduced fecal output and thin appearance, and pathological findings indicative of gastrointestinal tract irritation were seen in the animals killed or dying intercurrently. A no-effect level for maternal toxicity was, therefore, not established.

Abortions by one dam in the 1.0 mg/kg group, four in the 1.5 mg/kg group, and five in the 2.0 mg/kg group were considered evidence of fetotoxicity. Although non-treatment-related spontaneous abortion does occur, the single incidence seen in the 1.0 mg/kg group was not dismissed as being incidental to treatment in view of the incidences in the 1.5 and 2.0 mg/kg groups. In addition, one dam in each of the 1.0 and 1.5 mg/kg groups totally resorbed their litters.

Percentage pre-implantation loss was higher than controls in all paraquat treated groups with a consequent reduction in the mean number of implantations, mean number of live fetuses, mean gravid uterus weight, and mean litter weight, although mean fetal weight was comparable to controls. It is possible that the increased pre-implantation loss represented an effect of paraquat at an early stage of pregnancy, which could not be detected at termination. This loss may be associated with the incidence of abortions and total resorptions.

There were no treatment-related increases in the overall incidences of minor defects or variants. However, there were increases related to specific variants, including an increased percentage of fetuses with asymmetrical development of the pelvic girdle in the 2.0 mg/kg group and an increased percentage of fetuses with 27 pre-sacral vertebrae and extra 13th ribs in all treated groups, with the latter associated with the presence of extra thoracic ribs. These lesions represent fetotoxicity.

There was no treatment-related increase in the overall incidences of major defects. The major head lesions seen in one fetus in the 1.5 mg/kg group and two fetuses in the 2.0 mg/kg group were not identical and likely to be congenital in origin. Limb defects were seen in three fetuses in the 1.5 mg/kg group and in one fetus in the 1.0 mg/kg group, but there were none in the 2.0 mg/kg group. The 84% overall pregnancy rate in this study was satisfactory in this study, but insufficient numbers of litters were available for evaluation due to the treatment-related mortalities, total resorptions, and abortions in the treated groups. Therefore, the teratogenic potential of paraquat could not be assessed definitively.

It was concluded that administration of paraquat at dose levels of 1.0, 1.5 and 2.0 mg paraquat ion/kg bw/day resulted in overt maternal toxicity and fetotoxicity and that the maximum tolerated dose was exceeded at all dose levels. The number of dams and litters

available for assessment was too low, due to mortalities in all paraquat-treated groups, to allow an adequate assessment of the teratogenic potential of paraquat.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Maternal toxicity appeared evident at all treatment levels as death/moribundity, abortion, abnormal clinical signs (thin appearance, few or no feces, or abnormal feces), weight loss or decreased weight gain, decreased food consumption, gross stomach lesions and fluid colon contents. **Therefore, under the conditions of this study, the maternal LOAEL for paraquat dichloride in rabbits dosed on days 7-19 of gestation (with day of insemination = GD 1) was 1.0 mg/kg bw/day, based on death, abortion, weight loss, decreased food consumption, and gross pathology of the stomach and colon. A maternal NOAEL was not identified.**

2. **Developmental toxicity:** Developmental toxicity was evident at all of the evaluated dose levels.

   a. **Deaths/resorptions:** Maternal treatment corresponded with deaths at all treatment levels, including abortions in all treatment groups and total litter resorptions in the 1.0 and 1.5 mg/kg groups. The increased preimplantation loss across treatment groups probably represented a treatment-related effect at an early stage of pregnancy, i.e., very early intrauterine deaths (resorptions). The increased mean percentage of postimplantation loss and increased percentage of dams with early intrauterine deaths in the 1.5 mg/kg group also may have been treatment-related. The high maternal mortality in the 2.0 mg/kg group (in total, six deaths/humane sacrifices) and the greater number of abortions in this group have interfered with the expression of a dose response for these endpoints. However, it is also possible that the differences in preimplantation loss are related to animal husbandry issue(s), a problem with insemination technique (including the timing of the HCG injection), or compromised animal health.

   b. **Altered growth:** Maternal treatment did not affect fetal body weight, and there was no evidence of altered ossification rates in the treated groups compared to controls.

   c. **Developmental variations:** Maternal treatment at all dose levels increased the incidence of "any extra 13th rib." Maternal treatment at 2.0 mg/kg bw/day also increased the incidence of "27 pre-sacral vertebrae" and "27 pre-sacral vertebrae with any extra 13th rib." Although treatment-related, these findings are not considered adverse.

   d. **Malformations:** The total litter and fetal incidences of major defects were not significantly increased by treatment, and all individual malformations occurred at very low incidences. However, the number of litters and fetuses available for assessment was too low to assess malformations in a definitive manner, particularly in the 1.5 and 2.0 mg/kg groups. It is noted that across the treated groups there were three litters (four fetuses) that had major craniofacial and/or neural tube defects and four litters (four fetuses) that had major limb defects with none seen in the larger number of available control litters. While the incidences of these findings are too low to definitively attribute them to treatment, the sample size here is also small. The findings were not

identical across fetuses in this study and did not repeat the microphthalmia seen at the 1.5 and 2.0 mg/kg treatment-levels in the earlier definitive study (MRID 49009504). However, external abnormalities observed in a range-finding study (MRID 49009503) included meningocele in one 2.5 mg/kg fetus and multiple co-occurring major defects of the head and limbs in one 1.0 mg/kg fetus (flexure of both fore-paws, left anophthalmia, left ear attached back to front, heart exposed and exencephaly). The timing and type of the altered developmental events across the three studies is notable.

Therefore, under the conditions of this study, the developmental LOAEL for paraquat dichloride in rabbits dosed on days 7-19 of gestation (with day of insemination = GD 1) was 1.0 mg/kg bw/day, based on abortion and total litter resorption. The developmental NOAEL was not identified.

3. **Further discussion:** In this study there were no NOAELs for maternal or developmental toxicity because, although dose selection was identical to that used in MRID 49009504, the maternal toxicity evidenced in the current study was much greater. This raises a question on the conduct of the study, particularly in light of the absence of a Quality Assurance statement documenting observations of correct performance of the study procedures.

   The documented evidence of problems with prior studies, including inadvertent usage of test material that contained an emetic (adulterated test material) in MRID 49009502, poor pregnancy rates of 40-65% in all groups used in MRID 49009504, and a pregnancy rate of just 10% in the control group used in MRID 49009502, provide additional grounds for concern.

   For these reasons, the study is classified as **Unacceptable.**

C. **STUDY DEFICIENCIES:** Several major study deficiencies were identified. These included the following:

- Individual data were not provided.
- The study was not conducted in compliance with the Principles of Good Laboratory Practice.
- No Quality Assurance statement was provided.

The study is classified as **Unacceptable** because of these deficiencies, together with the fact that the unexplained disparity between the maternal toxicity seen in this study (MRID 49009505) and the previously conducted definitive study (MRID 49009504) calls into question whether or not there was a problem with animal health or the manner in which the study was conducted.

APPENDIX I: Prenatal Developmental Toxicity Study - Rabbit; Range-finding

TEST MATERIAL (PURITY): Paraquat dichloride technical:
CTL reference no. Y00061/159: paraquat ion (% not reported) and emetic PP796 (% not reported)
CTL reference no. Y00061/160: 33.6% w/w paraquat ion (as reported in MRID 49009503)

CITATION: Hodge, M. (1990) Paraquat - embryotoxicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/L/3423, October 18, 1990. MRID 49009502. Unpublished.

In a preliminary developmental toxicity study (MRID 49009502) paraquat dichloride technical was administered by gavage in deionized water to groups of ten artificially inseminated female New Zealand White rabbits at dose levels of 0, 2.5, 5, 10, or 20 mg/kg bw/day (dosing volume not reported) on gestation days (GDs) 7 through 19, inclusive, with the day of insemination designated as GD 1. For the first three days of dosing, CTL reference no. Y00061/159 (containing emetic PP796) was used in error; CTL reference no. Y00061/160 (33.6% w/w paraquat ion and *not* containing emetic) was used for the remainder of the dosing interval. On GD 30, dams were sacrificed and necropsied. Gravid uterine weights and the numbers of live fetuses and "intrauterine deaths" were recorded. Live fetuses were weighed, killed, subjected to external examination (to include checking for cleft palate), and discarded without further examination. With the exception of maternal wastage and the number of pregnancies, no data were provided in the study report.

Excessive mortality occurred at all treatment levels (40%-100%). Three 20-mg/kg females and one 10-mg/kg female were found dead on GD 10, and the remaining 20- and 10-mg/kg females and all 5-mg/kg females were sacrificed *in extremis* between GDs 9 and 15. Four 2.5 mg/kg females were sacrificed *in extremis* on GDs 16-17, and one 2.5 mg/kg female was sacrificed due to abortion on GD 22. The reported treatment-related clinical signs were subdued behavior and few/no feces. There was treatment-related weight loss during dosing; however, the surviving 2.5 mg/kg animals gained a substantial amount of weight once dosing ceased. Pregnant 2.5 mg/kg animals had slightly reduced food consumption during dosing. Treatment-related gross necropsy findings included sloughing of the gastric mucosa and hemorrhagic areas in the stomach. **Under the conditions of this study, which included dosing for three days with test material that contained an emetic, the maternal lowest-observed-adverse-effect level (LOAEL) for paraquat dichloride in rabbits treated on days 7-19 of gestation (with GD 1 = day of insemination) is 2.5 mg/kg bw/day, based on mortality, weight loss, and decreased food consumption. The maternal NOAEL is not identified.**

The numbers of pregnancies for the 0-, 2.5, 5-, 10-, and 20-mg/kg groups were 1, 6, 4, 2 and 6, respectively. It is unknown to the reviewer how many 2.5 mg/kg litters were available at the scheduled necropsy/cesarean sections. The study author stated that there was no evidence of any adverse effect on the number or survival of the young *in utero* or on mean fetal weight and that no external fetal abnormalities were seen, with the caveat that the litter and fetal data were very limited due to the small proportion of pregnant animals. **In the opinion of the reviewer, it would be inappropriate to use the results of this study to identify even a tentative**

developmental LOAEL and/or NOAEL.

Based on these results, the study author concluded that even the lowest dose used (2.5 mg/kg bw/day) clearly exceeded the maximum tolerated dose of the pregnant rabbit.

This study in rabbits is a range finding study and it is classified **Unacceptable/non-Guideline** and *does not satisfy* the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) in the rabbit.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. According to the GLP Compliance Statement, the study was not conducted in compliance with OECD Principles of Good Laboratory Practice (1997). A Quality Assurance statement was not provided, and the original title page stated, "The data in this report have not been quality assured."

APPENDIX II: Prenatal Developmental Toxicity Study - Rabbit; Range-finding

TEST MATERIAL (PURITY): Paraquat dichloride technical (33.6% w/w paraquat ion)

CITATION: Tinston, D. and J. Barber (1991) Paraquat - second embryotoxicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2730, July 11, 1991. MRID 49009503. Unpublished.

In a preliminary developmental toxicity study (MRID 49009503) paraquat dichloride technical (33.6% w/w paraquat ion; CTL reference no. Y00061/160) was administered by gavage in deionized water to groups of eight time-mated female New Zealand White rabbits at dose levels of 0, 1.0, 1.5, 2.0, or 2.5 mg/kg bw/day (dosing volume 1 mL/kg bw) on gestation days (GDs) 7 through 19, inclusive, with the day of insemination designated as GD 1. On GD 30, dams were sacrificed and necropsied. Gravid uterine weights, corpora lutea counts, and the numbers of live fetuses and early and late intrauterine deaths were recorded. Live fetuses were weighed, killed, subjected to external examination (to include checking for cleft palate), and discarded without further examination.

Mortality occurred in most groups, with 7, 6, 8, 6, and 4 animals surviving to scheduled termination in the 0-, 1.0, 1.5, 2.0, and 2.5 mg/kg groups, respectively. One 2.5 mg/kg female died on GD 15, and one female from each of the 0-, 1.0 , and 2.0 m/kg groups was sacrificed *in extremis* (on GDs 18, 16, and 22, respectively). Sacrifices due to abortion occurred in the 1.0 mg/kg group (1 female, GD 22), 2.0 mg/kg group (1 female, GD 24), and 2.5 mg/kg group (3 females, GDs 19-29), and the higher number of abortions in the 2.5 mg/kg group was considered treatment-related. Animals in the 2.0 and 2.5 mg/kg groups had increased incidences of few/no feces and thin appearance. During dosing, body weight gain was decreased in the 2.0 and 2.5 mg/kg groups, with the 2.5 mg/kg group showing a mean body weight loss (mean bw changes at 0, 2.0, and 2.5 mg/kg bw/day: 264.3, 10.2, and -2.5 g, respectively; n.s.). However, during the post-dosing interval, surviving animals in the 2.0 and 2.5 mg/kg groups had increased weight gains relative to controls (+69% and +77%, respectively; $p<0.05$ for 2.5 mg/kg group). The 2.5 mg/kg animals had decreased food consumption during treatment relative to controls (-72%; $p<0.05$). There were other large differences in food consumption for some of the treated groups during the treatment and/or post-treatment intervals, but these were of smaller magnitude than pre-treatment differences. Treatment-related gross necropsy findings included abnormal stomach contents in two 2.0 and three 2.5 mg/kg animals, sloughing of the gastric mucosa when washed in two 2.5 mg/kg animals, and hemorrhagic areas in the stomach in one 2.5 mg/kg animal. **Under the conditions of this study, based on decreased body weight gain and gross lesions (abnormal stomach contents), the maternal lowest-observed-adverse-effect level (LOAEL) for paraquat dichloride in rabbits treated on days 7-19 of gestation (with day of insemination = GD 1) is 2.0 mg/kg bw/day. The maternal NOAEL is 1.5 mg/kg bw/day.**

At cesarean, there were a total of 7, 5, 6, 5, and 4 litters evaluated in the 0-, 1.0, 1.5, 2.0, and 2.5 mg/kg groups, respectively. There were no total litter resorptions and no clear effects on intrauterine parameters were seen. External abnormalities included one 1.0 mg/kg fetus with major abnormalities consisting of flexure of both fore-paws, left anophthalmia, left ear attached back to front, heart exposed and exencephaly and one 2.5 mg/kg fetus with meningocele. These were not attributed to treatment due to their low incidences. **Under the conditions of this**

study, based on increased abortions, the developmental LOAEL for paraquat dichloride in rabbits treated on days 7-19 of gestation (with day of insemination = GD 1) is 2.5 mg/kg bw/day. The developmental NOAEL is 2.0 mg/kg bw/day.

Based on these results, dose levels of 0, 1.0, 1.5, and 2.0 mg/kg bw/day (on GD 7-17, inclusive) were selected for the main study.

This study in rabbits is a range finding study and it is classified **Unacceptable/non-Guideline** and *does not satisfy* the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) in the rabbit.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. According to the GLP Compliance Statement, the study was not conducted in compliance with OECD Principles of Good Laboratory Practice (1997). A Quality Assurance statement was not provided, and the original title page stated, "The data in this report have not been quality assured."

APPENDIX III: Prenatal Developmental Toxicity Study - Rabbit; Range-finding

TEST MATERIAL (PURITY): Paraquat technical liquor (33.6% w/w paraquat ion)

CITATION: Tinston, D. (1991) Paraquat - teratogenicity study in the rabbit (final report). ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2749, September 26, 1991. MRID 49009504. Unpublished.

In a developmental toxicity study (MRID 49009504) paraquat technical liquor (33.6% w/w paraquat ion; Lot/batch #YF6219 [ex No. 9 Product Stock Tank]) was administered by gavage in deionized water to twenty inseminated female New Zealand White rabbits/dose by gavage at dose levels of 0, 1.0, 1.5, or 2.0 mg/kg bw/day (dose volume 1 mL/kg bw) on gestation days (GDs) 7 through 19, inclusive, with the day of insemination designated as GD 1. On GD 30, surviving does were sacrificed and necropsied. Gravid uterine weights, corpora lutea counts, and the numbers and positions of live fetuses and early and late intrauterine deaths were recorded. Fetuses were weighed, examined for external anomalies (to include cleft palate), sexed internally, subjected to visceral examination, and fixed in methanol. Following 24 hours fixation, the head of each fetus was cut along the fronto-parietal suture line, and the brain was examined, with the carcass subsequently processed for and subjected to skeletal examination.

One 1.0 mg/kg female was found dead on GD 10 without preceding abnormal clinical signs, and one 1.5 mg/kg female was sacrificed *in extremis* on GD 22. Two 2.0 mg/kg females were sacrificed due to abortion on GDs 24-25. Animals in the 1.5 and 2.0 mg/kg groups had increased incidences of few/no feces. Treatment-related effects on body weight gain included mean body weight loss in 1.5 and 2.0 mg/kg females during GDs 7-10 (mean bw changes at 0, 1.5, and 2.0 mg/kg bw/day: +35.5, -85.1, and -52.9 g, respectively; $p<0.01$) which, together with decreased body weight gain during GDs 10-13 (71% to 75% less than controls; n.s.) resulted in decreased overall body weight gain for these groups over the GD 7-19 dosing interval (both: -56%; $p<0.05$). Mean food consumption of the 1.5 and 2.0 mg/kg females was significantly decreased ($p<0.01$) during GDs 7-10 (40-41% less than controls) and GDs 10-13 (-36%) and slightly decreased during GDs 13-16 (14-15% less than controls; n.s.), with compensatory increases seen during GDs 19-22 for 1.5 mg/kg females (+19%; $p<0.05$) and during days 19-30 for 2.0 mg/kg females (+13-17%; $p<0.05$). Gross necropsy findings included single incidences of various stomach lesions, including abnormal contents (at 1.5 mg/kg bw/day), sloughing of the gastric mucosa when washed (at 1.0, 1.5, and 2.0 mg/kg bw/day), hemorrhagic areas (at 1.0 and 2.0 mg/kg bw/day), and thin wall (at 2.0 mg/kg bw/day); none of these were seen in controls. **The maternal lowest-observed-adverse-effect level (LOAEL) for paraquat dichloride in rabbits treated on days 7-19 of gestation (with day of insemination = GD 1) is 1.5 mg/kg bw/day, based on body weight loss, decreased body weight gain, and decreased food consumption. The maternal NOAEL is 1.0 mg/kg bw/day.**

At necropsy/cesarean section, there were no total litter resorptions, and a total of 92 (12), 57 (10), 59 (8), and 64 (11) fetuses (and litters) were evaluated in the 0-, 1.0, 1.5, and 2.0 mg/kg groups, respectively. No clear treatment-related effects on intrauterine parameters were seen. The 1.0 and 2.0 mg/kg dams (but not the 1.5 mg/kg dams) had non-statistically significant increases in mean percentage preimplantation loss (24.4%, 30.1%, 12.7%, and 30.5% for all groups, in ascending dose order) and postimplantion loss (6.0%, 15.6%, 8.8%, and 16.9%), with

corresponding reductions in mean numbers of implantations (8.25, 7.00, 8.13, and 6.91) and live fetuses (7.67, 5.70, 7.38, and 5.82). The decreased litter sizes resulted in reduced mean gravid uterine weights and litter weights in these same groups, but mean fetal weights of the treated groups were comparable to controls. The mean litter proportion of male fetuses in the 2.0 mg/kg group was higher than controls (62.6% vs. 44.4%; $p<0.05$) because 3/8 dams had litters of males only (5, 2, and 1 male respectively). However, the overall percentage of male fetuses in the 2.0 mg/kg group was only 53.1% (vs. 45.7% for controls).

Major defects were observed in 2 (2), 0, 2 (1), and 2 (2) fetuses (and litters) in the 0-, 1.0, 1.5, and 2.0 mg/kg groups, respectively. These included a cardiac anomaly with the aorta extremely reduced in one 1.5 mg/kg fetus, unilateral microphthalmia in one 1.5 mg/kg fetus, and bilateral microphthalmia in two 2.0 mg/kg fetuses from separate litters. The occurrences of microphthalmia may be treatment-related, but this cannot be determined definitively at these very low incidences. There was a dose-related increase in the mean percentage of fetuses with minor skeletal defects (33.3%, 43.2%, 45.5%, and 63.5%; $p<0.05$ at 2.0 mg/kg bw/day), and the 2.0 mg/kg group also had a statistically significant increase in the litter incidence of the minor defect "2nd cervical vertebrae - arch partially ossified" (36.4% of litters vs. 0% of control litters; $p<0.05$). At 2.0 mg/kg bw/day, the litter incidence of the variant "27 pre-sacral vertebrae" was significantly increased (100% vs. 50% of control litters; $p<0.01$). The incidences of the variants "extra 13th normal-length ribs" and "any extra 13th rib" were increased by treatment. The fetal incidences of "extra 13th normal-length ribs" were significantly increased ($p<0.01$) at 1.5 and 2.0 mg/kg bw/day (27.2%, 50.8%, and 67.2% for 0-, 1.5 , and 2.0 mg/kg groups, respectively, with 83%-100% of litters affected), and the fetal incidence of "any extra 13th rib" was significantly increased at 2.0 mg/kg bw/day (78.1% vs. 50.0% for controls, with 92%-100% of litters affected). Fetal ossification did not appear to be affected by treatment, as evidenced by the absence of treatment-related differences in the *manus* or *pes* scores.

Although not treatment-related, it must be noted that the pregnancy rates attained in this study were low: 60%, 55%, 40%, and 65% for the 0-, 1.0, 1.5 and 2.0 mg/kg groups, respectively, with an overall pregnancy rate of 55%. This resulted in just 12, 10, 8, and 11 litters available for evaluation in these same respective groups.

Under the conditions of this study, the developmental LOAEL for paraquat dichloride in rabbits treated on days 7-19 of gestation (with day of insemination = GD 1) is 2.0 mg/kg bw/day, based on abortions. The developmental NOAEL is 1.5 mg/kg bw/day.

The low pregnancy rates attained in this study resulted in fewer than twelve litters available for evaluation in all treated groups. The numbers of litters were considered insufficient to adequately to fulfill the objectives of the study, and a second definitive study (MRID 49009505) was initiated, using the same dose levels.

This developmental toxicity study in the rabbit due to the deficiencies cited is classified **Unacceptable/Guideline** and *does not satisfy* the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) in the rabbit.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. According to the GLP Compliance Statement, the study was not conducted in compliance with OECD Principles of Good Laboratory Practice (1997). A Quality Assurance statement was not

provided, and the original title page stated, “The data in this report have not been quality assured.”

APPENDIX IV: Testing Laboratory's Classification of Fetal Observations [a]

Within CTL, fetal observations are classified as major defects, minor defects, or variants for the purposes of quantifying the severity of a particular lesion and assessing the significance to the animal. The classifications are not fixed but are dependent on species and strain of animal.

The category assigned to an observation is based on the following guidelines:

Major defects: Permanent structural or functional deviations that are considered likely to be incompatible with survival or are rarely seen. A complex association of minor observations may also be ascribed this classification.

Minor defects and variants: Small, generally transient deviations which are considered not to be incompatible with life and which frequently represent a manifestation of delayed development, e.g., reduced ossification.

The "minor defect" classification is used for observations which generally occur at low frequency, in less than 10% of the control population. The "variant" classification is used for observations which consistently occur at a frequency greater than 10%.

Temporal shifts are known to occur in the frequency of some observations and this is also taken into account at the time of classification.

[a] Taken from Appendix G, p. 83, MRID 49009505.

APPENDIX V: Scale Used for Assessment of Skeletal Ossification of the *Manus* and *Pes* [b]

Scale	Meaning
l. (good)	Metacarpals/metatarsals and 1st, 2nd and 3rd rows of phalanges fully ossified. In practice it is the 2nd row of phalanges which show most variation. To be regarded as fully ossified they should be clearly triangular in shape.
2.	Metacarpals/metatarsals, 1st and 3rd rows of phalanges fully ossified. 2nd row of phalanges fully ossified with the exception of the 5th digit, which is partially ossified.
3.	One metacarpal/metatarsal partially ossified remainder fully ossified. 1st, 2nd and 3rd row of phalanges fully ossified with the exception of the 5th digit, which is partially ossified.
4.	One metacarpal/metatarsal partially ossified, remainder fully ossified. 1st and 3rd rows of phalanges fully ossified. Some of the 2nd row may be partially or not ossified.
5.	One metacarpal or metatarsal not ossified, remainder fully ossified. 1st and 3rd rows of phalanges mainly ossified but a few partially ossified. 2nd row of phalanges may be partially or not ossified.
6. (poor)	One metacarpal or metatarsal not ossified, remainder fully ossified. 2nd row of phalanges not ossified, occasionally phalanges in 1st and 3rd row not ossified, remainder partially ossified.

[b] Taken from Appendix F, p. 82, MRID 49009505.

DATA EVALUATION RECORD

PARAQUAT

NON-GUIDELINE, USING MODIFIED TEMPLATE FOR OCSPP 870.3150

STUDY TYPE: MEDIAN LETHAL DOSE AND RADIOLABELING STUDY OF DISTRIBUTION AND EXCRETION – ORAL GAVAGE DOSING, FEMALE RABBITS

MRID 49009501

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by
Summitec Corporation
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Task 6-101

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.
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EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** July 16, 2014
EPA Work Assignment Manager: Lori Brunsman **Signature:**
Science Info. Mgmt. Branch, Health Effects Division (7509P) **Date:** 7/17/14

Template version 09/11

TXR#: 0056764

DATA EVALUATION RECORD

STUDY TYPE: Acute oral toxicity study and radiolabeling study of distribution and excretion [oral gavage dosing]-[female rabbits]; Non-guideline.

PC CODE: 061601 **DP BARCODE:** D409213

TEST MATERIAL (PURITY): Paraquat dichloride (paraquat); 33.0% paraquat ion (w/w) in the stock solution

SYNONYMS: 1,1′-dimethyl 4,4′-bipyridilium

CITATION: Farnworth, M., Foster, J., and Lock, E. (1993). Paraquat—The toxicity of paraquat to rabbits following oral administration—Final Report. Zeneca Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, United Kingdom. CTL Study Numbers: XB2434, XB2567, XB2607, and XB2610. Report Number CTL/R/1164. September 20, 1993. MRID 49009501. 38 pages. Unpublished.

SPONSOR: Syngenta Crop Protection, LLC, 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419-8300.

EXECUTIVE SUMMARY:

In an acute oral toxicity/Median Lethal Dose study (MRID 49009501), paraquat dichloride (33.0% paraquat ion (w/w) in stock solution, batch YF6219,) was administered by gavage to groups of 2 female New Zealand rabbits at dose levels of 2, 4, 8, 12, 16, 20, 24, or 30 mg paraquat ion/kg bw, or to groups of 4 female New Zealand rabbits at dose levels of 40 or 50 mg paraquat ion/kg bw. Animals were observed for up to 10 days unless they had been sacrificed before scheduled termination. In two Tissue Distribution and Excretion experiments [^{14}C]-methyl labeled paraquat dichloride (33.0% paraquat ion (w/w) in stock solution, (a mixture of unlabeled and radiolabeled paraquat) was administered by gavage to 20 female New Zealand rabbits at a dose level of 30 mg paraquat ion/kg bw, with 5 animals being sacrificed after 1, 4, 24, and 48 hours. In a third Tissue Distribution and Excretion experiment [^{14}C]-labeled paraquat dichloride was administered by gavage to groups of 4 female New Zealand rabbits at dose levels of 0, 2, or 30 mg paraquat ion/kg bw, with paraquat-treated animals being sacrificed after 144 and 72 hours at the lower and higher doses, respectively.

In the Median Lethal Dose study, rabbits receiving up to 12 mg paraquat ion/kg bw showed no signs of toxicity over the 10-day period of observation. Higher doses resulted in body weight loss, decreased food consumption and some hematuria. All four animals receiving a single oral dose of 50 mg paraquat ion/kg bw died during the observation period. It was concluded that the median

lethal dose is between 40 and 50 mg paraquat ion/kg bw following a single oral treatment. Microscopic pathology of the kidneys of the animals with azotemia showed multifocal hydropic change in the S2 segment of the proximal tubules. The three Tissue Distribution and Excretion experiments showed that the peak concentration in blood plasma was reached within one hour after treatment and that the concentration rapidly returns to near zero following treatment. No toxic effects were associated with the single dose of 2 mg paraquat ion/kg bw. At both doses (2 or 30 mg/kg), only about 10% of the oral dose was absorbed, and it was excreted in the urine. At the higher dose, both creatinine and urea levels in urine far exceeded the normal range at 2 and 3 days after treatment. While the lower dose of paraquat had no effect on urinary output, the higher dose reduced the urine flow by about 50% over the duration of the study and also produced a marked reduction in fecal output. As a result of the reduced urine and fecal outputs, only a small proportion of the administered dose was eliminated by these routes during the 72 hours study. At the oral dose of 30 mg paraquat ion/kg bw, there was both functional and morphological renal injury, which was thought to contribute to the mortality observed at higher doses. Microscopic pathology of the kidneys showed signs of multifocal hydropic change in the S_2 segment proximal tubules, interstitial fibrosis, multifocal tubular necrosis in the S_2 segment proximal tubules, tubular dilation, luminal casts, interstitial nephritis, and interstitial fibrosis. Histological examination of stomachs, duodenums, livers, and lungs of these same animals showed no compound-related effects, with the possible exception of one animal that, at 72 hours after dosing, had submucosal edema of the stomach wall, squamous metaplasia of the mucosa, and mucosa atrophy of the stomach. None of the 3 control animals showed any abnormalities in these organs in the microscopic pathology examination. There was no testing in this study of effects on hematology, most clinical chemistry parameters, organ weights, or gross and histologic pathology in most organs and tissues typically studied.

The LOAEL is a single oral dose of 30 mg paraquat ion/kg bw based on renal damage revealed by azotemia and by microscopic pathology findings of multifocal hydropic change in the S_2 segment of the proximal tubules and additional renal damage. No NOAEL is indicated because of the limited testing of only 2 animals at all but one of the lower doses tested. While no signs of toxicity were seen in any animals receiving single oral doses as high as 12 mg paraquat ion/kg bw, some animals at the doses of 16, 20, and 24 mg paraquat ion/kg bw exhibited loss of appetite, and one animal at the 24 mg paraquat ion/kg bw dose had unspecified loss of weight and hematuria on day 8 of observation. The data are considered too sparse at the doses of 16, 20, and 24 mg paraquat ion/kg bw to provide the basis for a LOAEL.

This acute oral toxicity/median lethal dose and tissue distribution and excretion study in the rabbit is **Acceptable (non-guideline)**. This non-guideline study provided useful information about the toxic effects of paraquat when administered orally as a single dose to female rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. According to those statements, the study was not conducted in compliance with OECD Principles of Good Laboratory Practice (1997), and the data in the report were not quality assured.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Paraquat dichloride

Description: Dark brown/black liquid

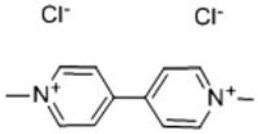
Lot/batch #: YF6219 (as unlabeled is CTL Reference No. Y00061/160 and as [^{14}C] methyl labeled is CTL Reference No. Y00061/185/001). The position of the radiolabel was not reported.

Purity: 33.0% paraquat ion (w/w) in the stock solution. The radiolabeled solution had a specific activity of 93 mCi/mMole and radiochemical purity of 94%. It was supplied by Cambridge Research Biochemicals Ltd., Billingham, Cleveland.

Compound stability: Not provided

CAS # if TGAI: 1910-42-5

Structure:



2. Vehicle and/or positive control: Deionized water

3. Test animals:

Species: Rabbit (only females)

Strain: New Zealand

Age/weight at study initiation: Age not stated / Body weight ~4 kg

Source: Controlled Animal Breeding Unit at Alderley Park

Housing: Individually in rabbit metabolism cages in acclimatized animal rooms; cages were made of aluminum with plastic flooring; dimensions: height, 45 cm; width, 48 cm; depth, 60 cm.

Diet: BEEKAY Rabbit Maintenance Diet supplied by Bantin and Kingman Ltd., Grimston, Aldbrough, Hull, North Humberside, United Kingdom. Diet formation and specification details were provided together with analyses of the batches used—no contaminants were found that were thought to be at sufficient concentration to affect the outcome of the study, *ad libitum*

Water: In animal drinking bottles, *ad libitum*

Environmental conditions:
- **Temperature:** 15-19 °C
- **Humidity:** 40-70% relative humidity
- **Air changes:** 25-30/hr
- **Photoperiod:** 12 hrs dark/ 12 hrs light

Acclimation period: At least 7 days in animal rooms; ~24 hours in the metabolism cages immediately prior to dosing in distribution and excretion experiments.

B. STUDY DESIGN:

1. In life dates: Start: Not provided; End: Not provided

2. Animal assignment: Animals were assigned to the test groups noted in Table 1. There was no mention of any randomization procedure.

TABLE 1A: Study design in Median Lethal Dose Experiment No. XB2434

Test group	Oral dose to animal (mg paraquat ion/kg bw)	# Female
Control	0	0
Low	2	2
Mid 1	4	2
Mid 2	8	2
Mid 3	12	2
Mid 4	16	2
Mid 5	20	2
Mid 6	24	2
Mid 7	30	2
Mid 8	40	4
High	50	4

TABLE 1B: Study design in Tissue Distribution and Excretion Experiment XB2567

Test group	Dose to animal (mg of partially-radiolabeled paraquat ion/kg bw)	# Female	Termination (Hours)
Control			
Experimental	30	5	48

TABLE 1C: Study design in Tissue Distribution and Excretion Experiment XB2610

Test group	Dose to animal (mg of partially-radiolabeled paraquat ion/kg bw)	# Female	Termination (Hours)
At 1 hour	30	5	1
At 4 hours	30	5	4
At end	30	5	24

TABLE 1D: Study design in Tissue Distribution and Excretion Experiment XB2607

Test group	Dose to animal (mg of partially-radiolabeled paraquat ion/kg bw)	# Female	Termination (Hours)
Control	0	4	
Low	2	4	144
High	30	4	72*

*Animals were sacrificed at 72 hours because of the progressive increase in creatinine and urea in the blood plasma.

3. **Dose selection rationale:** The dose levels chosen in the Median Lethal Dose experiment were influenced by examination of available literature and by the type of experiment. The dose levels in the other experiments were selected based on the results from the Median Lethal Dose experiment. Details of the preparation and application of the oral doses used are found in the Appendix of this review.

4. **Diet preparation and analysis:** The test article was not administered in the diet.

Results:
Homogeneity analysis: Not applicable

Stability analysis: Not applicable

Concentration analysis: Not applicable

5. **Statistics:** The area under the curve (AUC) for plasma paraquat was calculated using the trapezoid method. In addition to the numbers of animals that died or showed clinical signs or histopathological findings, the parameters analyzed were (a) paraquat concentrations at various times after treatment in liver, lung, kidney and blood plasma, (b) creatinine and urea concentrations in blood plasma, (c) urinary output, and (d) the percentages of the dose of paraquat that were excreted in the urine and the feces. Means and standard errors of the mean (SEM) were calculated and presented in tables or graphs. The Reviewer considers the analyses used to be appropriate.

C. METHODS:

1. **Observations:**

   1a. Cageside observations: Animals were inspected at least 4 times per day for signs of toxicity and mortality.

   1b. Clinical examinations: Clinical examinations were conducted at least 4 times per day.

2. **Body weight:** In the Median Lethal Dose experiment, animals were weighed prior to the administration of the single oral dose of paraquat and at several hours post dosing as required. In the three Tissue Distribution and Excretion experiments, all animals were weighed prior to dosing before being bled via a peripheral ear vessel. Animals were also weighed at 24, 48, and 72 hours after dosing except in those experiments where some or all animals were sacrificed before those times.

3. **Food consumption and compound intake:** Food consumption was monitored on a daily basis during each experiment.

4. **Ophthalmoscopic examination:** Eyes were not examined.

5. **Hematology and clinical chemistry:** Blood was collected prior to dosing by bleeding via the peripheral ear vessel. In the Median Lethal Dose experiment, blood was collected by cardiac puncture on termination. In all other experiments, blood samples of nominally 0.75 mL were collected via the peripheral ear vessel. Blood was collected prior to sacrifice from animals terminated at 1, 4, and 24 hours. Blood samples were taken at the following time points following dosing: 15 and 30 minutes and 1, 2, 4, 7, 12, 24, 48, 72, 96, 120, and 144 hours, depending on the termination time point. Animals were not fasted before collection of blood. The CHECKED (X) parameters were examined.

a. Hematology: Blood was not collected for hematology

	Hematocrit (HCT)*		Leukocyte differential count*
	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)*
	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)*
	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)*
	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry:

	ELECTROLYTES	X	OTHER
	Calcium*		Albumin*
	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
	Phosphorus*		Total Cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose*
	ENZYMES (more than 2 hepatic enzymes eg.,*)		Total bilirubin*
	Alkaline phosphatase (ALK)*		Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Plasma paraquat concentration
	Alanine amino-transferase (also SGPT)*		
	Aspartate amino-transferase (also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

Blood was centrifuged at ~1000 x g for 15 minutes at 4 ºC to collect the plasma, which was stored at 4 ºC before analysis. Plasma paraquat concentration was determined by radioimmunoassay. All plasma samples and a series of paraquat ion standards were buffered and mixed with triatiated (^{3}H)-paraquat. Antiserum raised against a derivative of monoquat was added. After ~15 minutes, any free paraquat ion was adsorbed onto a bovine serum albumin-charcoal suspension. After the solution was centrifuged at 1000 x g for 15 minutes at 4 ºC, the antibody-(^{3}H)-paraquat ion complex in the supernatant was counted in a liquid scintillation counter for 1 minute. The paraquat ion concentration in each sample was determined by comparison with the standards.

6. <u>Urinalysis and Fecal Analysis</u>: Urine and feces were collected from unfasted animals in the tissue distribution and excretion experiments. Urine was removed and weighed at 3, 7, 12, 24, 48, 72, 96, 120, and 124 hours after dosing, depending on the termination time point. Fecal samples were removed at 12, 24, 48, 72, 96, 120, and 144 hour after dosing, weighed, and frozen at -20 ºC until time of study. The CHECKED (X) parameters were examined in urine.

	Appearance*		Glucose*
X	Volume* and radioactivity of labeled paraquat		Ketones
	Specific gravity / osmolality*		Bilirubin
	pH*		Blood / blood cells*
	Sediment (microscopic)		Nitrate
	Protein*		Urobilinogen

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

7. <u>Sacrifice and pathology</u>: In the Median Lethal Dose experiment, animals were sacrificed by intravenously administered Euthatal at day 10 after treatment or earlier at the discretion of the study director. All tissues were examined by macroscopic observation. Kidneys of all animals killed prior to 10 days were submitted for histopathological examination. In the other experiments, all animals that died and those sacrificed on schedule, except for those sacrificed at 1 and 4 hours, were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. Approximately 1.5 g of the liver, lungs, and left kidney were removed for measurement of radioactivity. The remainder of the lungs, several samples of liver, and the stomach, duodenum, and right kidney were processed for light microscopic examination.

X	DIGESTIVE SYSTEM
	Tongue
	Salivary glands*
	Esophagus*
X	Stomach*
X	Duodenum*
	Jejunum*
	Ileum*
	Cecum*
	Colon*
	Rectum*
XX	Liver*+
	Gall bladder*+
	Pancreas*

X	RESPIRATORY
	Trachea*
XX	Lung*
	Nose*
	Pharynx*
	Larynx*

	CARDIOVASC./HEMAT.
	Aorta thoracic*
	Heart*+
	Bone marrow*
	Lymph nodes*
	Spleen*+
	Thymus*+

X	UROGENITAL
XX	Kidneys*+
	Urinary bladder*
	Testes*+
	Epididymides*+
	Prostate*
	Ovaries*+
	Uterus*+
	Mammary gland*

	NEUROLOGIC
	Brain*+
	Peripheral nerve*
	Spinal cord (3 levels)*
	Pituitary*
	Eyes (optic nerve)*

	GLANDULAR
	Adrenal gland*+
	Lacrimal gland
	Parathyroid*+
	Thyroid*+

	OTHER
	Bone (sternum and/or femur)
	Skeletal muscle
	Skin*
	All gross lesions and masses*

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150
\+ Organ weight required for non-rodent studies.

Duplicate 0.5 g trimmed slices of lung and liver and duplicate 0.4 g samples of a homogenate of the left kidney of each animal were transferred into pre-weighed glass scintillation vials and weighed. Soluene-350 was added to each vial, and the tissue was allowed to digest at ambient temperatures. Ten mL of Hionic Fluor were added to duplicate volumes of the solubilized tissues for liquid scintillation counting (LSC).

8. Methods: A Packard Tricarb model 2000CA instrument (Canberra Packard Ltd., Pangbourne Berks, United Kingdom) was used for all LSC. Samples together with the oxidized samples were counted for [^{14}C]-radioactivity to a 1% standard deviation of the count or for a maximum of 10 minutes each. Results were corrected for background activity and counting efficiency using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench correction data entered into the instrument computer. Sample vials were counted for [^{3}H] radioactivity for only 1 minute. The limit of detection of radioactivity measurements in these experiments was taken as 50 disintegrations per minute, which was twice the scintillation counter background rate.

Radioactivity in the urine and other solutions (including the dose preparation) was measured by direct LSC of replicate weighed samples each admixed with 10 mL of Optiphase 'MP' as scintillant. Fecal samples were freeze-dried and ground to a homogeneous powder using a coffee grinder. Radioactivity in 0.2 g replicates was determined by LSC following sample oxidation using a Packard Tricarb model 306 sample oxidizer. [^{14}C]-CO_2 generated on combustion of the samples was adsorbed into Caro-sorb E and mixed with Permafluor E^+ scintillant. Compensation was made for the efficiency of oxidation of test samples relative to [^{14}C]-standard oxidation efficiencies, which had been determined at regular intervals for each series of oxidations.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** In the Median Lethal Dose experiment, the pairs of animals receiving single oral doses of 2, 4, 8, or 12 mg/kg bw paraquat ion showed no signs of toxicity over the 10-day period of observation. One of the two animals that received 16 mg paraquat ion/kg bw showed some loss of appetite during the first 4 days after treatment. Lack of an appetite was more pronounced in the pairs of animals that received the 20 and 24 mg paraquat ion/kg bw doses. One of the animals given the higher of these two doses showed a loss of body weight as well as hematuria on day 8. Both of the animals given a single oral dose of 30 mg paraquat ion/kg bw refused to eat for the first 2 days. While one of those animals showed gradual improvement of appetite, the other one was terminated at day 3 because of subdued activity and moderate hematuria. The 4 animals given the single oral dose of 40 mg paraquat ion/kg bw stopped eating for the first few days and then gradually began to eat, but one of them died on day 3. Of the 4 animals given the single oral dose of 50 mg paraquat ion/kg bw, one died at least 3 days after treatment, one was sacrificed (when moribund) on day 3, and the remaining 2 were sacrificed on day 8 because they showed marked diarrhea, hypothermia, and general lethargy.

   In the Tissue Distribution and Excretion experiments, a dose of 2 mg paraquat ion/kg bw produced no signs of toxicity. The signs of toxicity following a single dose of 30 mg paraquat ion/kg bw were primarily lack of appetite, with little food or water consumption and a loss of body weight. The animals were sacrificed at 72 hours after dosing. Unlike in the Median Lethal Dose experiment, no details were provided as to when or if the animals began to eat and drink or how much weight they lost. However, at the high dose they were observed for a maximum of 3 days, instead of 10 days as in the Median Lethal Dose experiment.

2. **Mortality:** In the Median Lethal Dose experiment, there was no mortality during the 10-day period of observation in the pairs of animals receiving single oral doses of 2, 4, 8, 12, 16, 20, or 24 mg paraquat ion/kg bw. Of the two animals receiving a single oral dose of 30 mg paraquat ion/kg bw, one was terminated at day 3 because of subdued activity and moderate hematuria. One of four animals receiving a single oral dose of 40 mg paraquat ion/kg bw died on day 3. All four animals receiving a single oral dose of 50 mg paraquat ion/kg bw died during the observation period. It was concluded that the median lethal dose is between 40 and 50 mg/kg bw following a single oral treatment. In the Tissue Distribution and Excretion experiments, neither dose (2 or 30 mg paraquat ion/kg bw) resulted in mortality.

B. BODY WEIGHT AND WEIGHT GAIN:

Changes in body weight were only noted for a few animals in the Median Lethal Dose experiment. One of two animals receiving the 24 mg/kg bw dose experienced a loss of body weight. The 3 of 4 animals receiving the 30 mg/kg bw dose that survived the entire 10-day observation period experienced decreases in body weight of 11, 12, and 13%. No details were provided on changes in body weight in the Tissue Distribution and Excretion experiments.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** As noted above, the higher-dose oral treatments with paraquat caused the animals to lose their appetites and, in some cases, to stop eating entirely.

2. **Compound consumption**: Amounts of compound administered are shown in Table 1.

3. **Food efficiency:** Not calculated or required.

D. OPHTHALMOSCOPIC EXAMINATION: None conducted.

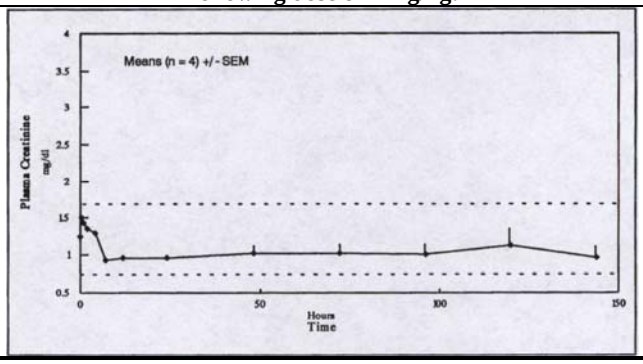
E. BLOOD ANALYSES:

1. **Hematology:** Not done.

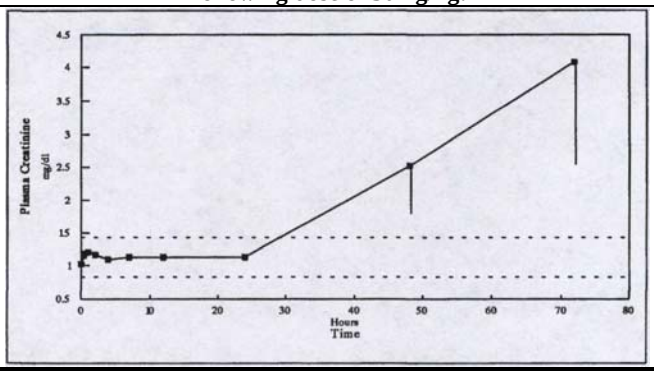
2. **Clinical chemistry:** In the Median Lethal Dose experiment, the post mortem blood samples showed that some of the animals receiving the high doses had elevations in plasma creatinine and urea. Figure 1 shows the plasma creatinine levels following a single oral dose of 2 or 30 mg paraquat ion/kg bw in the Distribution and Excretion experiments. Figure 2 shows the plasma urea levels following those same doses. The progressive increases in creatinine and urea correlate with the renal damage revealed in the microscopic pathology, which showed multifocal hydropic change in the S2 segment of the proximal tubules and additional renal damage as described in Section II.G.3.

Figure 1. Concentrations of creatinine in blood plasma of rabbits at various intervals following administration of the 2 and 30 mg paraquat ion/kg bw doses -- dashed lines show normal range.

Following dose of 2 mg/kg:



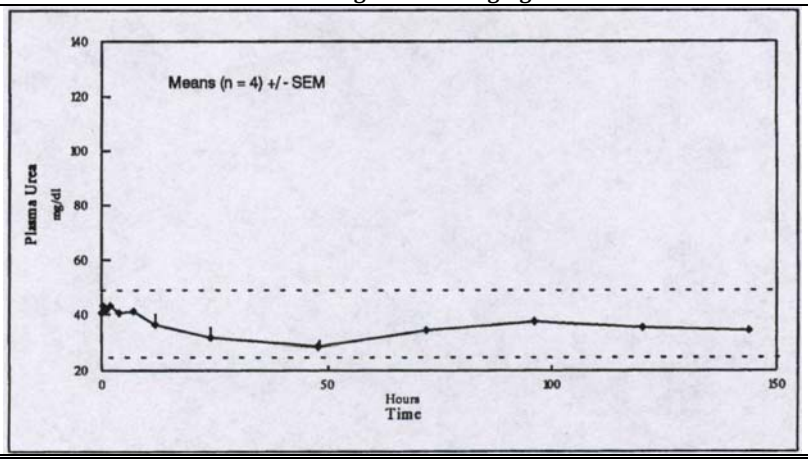
Following dose of 30mg/kg:



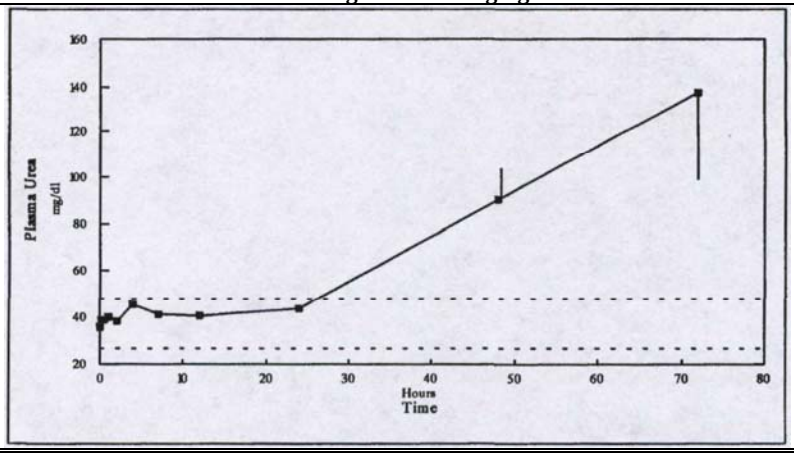
Data from Figure 3, p. 31 of 38, and Figure 7, p. 34 of 38, MRID 49009501.

Figure 2. Concentrations of urea in blood plasma of rabbits at various intervals following administration of the 2 and 30 mg paraquat ion/kg bw doses -- dashed lines show normal range.

Following dose of 2 mg/kg:



Following dose of 30mg/kg:

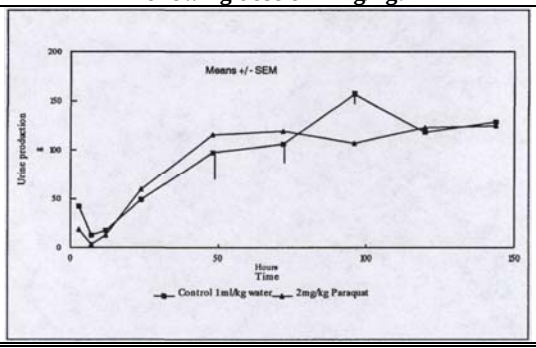


Data from Figure 3, p. 31 of 38, and Figure 7, p. 34 of 38, MRID 49009501.

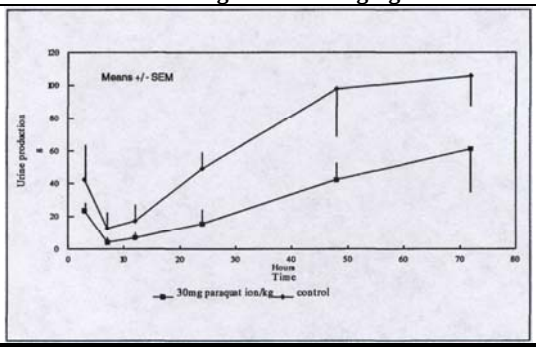
F. URINALYSIS AND FECAL ANALYSIS: Figure 3 shows the urinary outputs following a single oral dose of 2 or 30 mg paraquat ion/kg bw. Figures 4 and 5 show the percentages of the dose of paraquat excreted in the urine and feces following a single oral dose of 2 or 30 mg paraquat ion/kg, respectively. At the lower dose, 94% of the total dose was excreted over 7 days, of which about 7% was eliminated in the urine, with 6% of the total dose being eliminated by that route in the first 24 hours. The remainder of the dose was excreted in the feces, with about 60% of the total dose being eliminated by that route in the first 24 hours. While the lower dose of paraquat had no effect on urinary output, the higher dose reduced the urine flow by about 50% over the duration of the experiment and also produced a marked reduction in fecal output. As a result of the reduced urine and fecal outputs, only a small proportion of the administered dose was eliminated by these routes during the 72 hours studied. Urine and feces only accounted for elimination of 8% and 3% of the dose, respectively.

Figure 3. Urinary output in rabbits at various intervals following administration of the 2 and 30 mg paraquat ion/kg bw doses, in comparison to rabbits given drinking water at 1 mg/kg.

Following dose of 2 mg/kg:



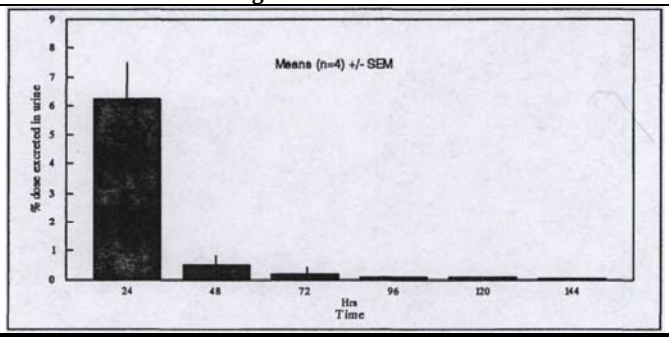
Following dose of 30mg/kg:



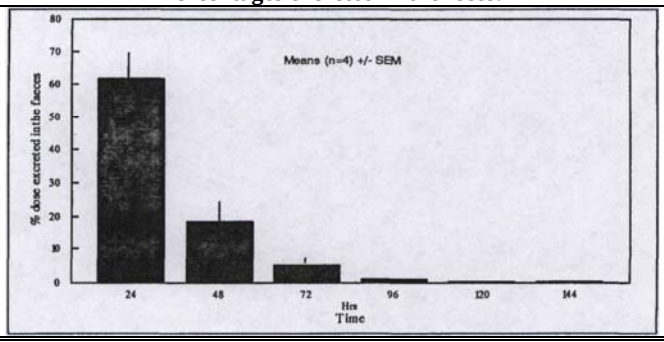
Data from Figure 4, p. 32 of 38, and Figure 9, p. 36 of 38, MRID 49009501.

Figure 4. Percentages of the dose of paraquat excreted in the urine and feces at various intervals following a single oral dose of 2 mg paraquat ion/kg bw.

Percentages excreted in the urine:



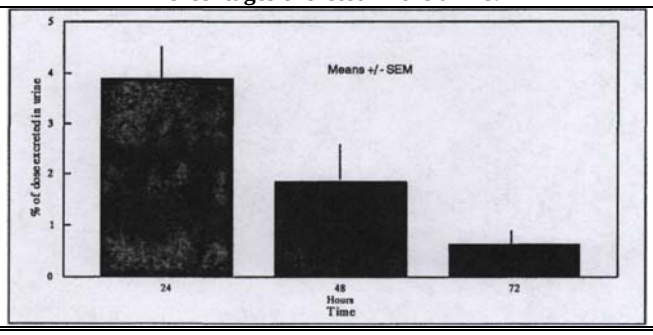
Percentages excreted in the feces:



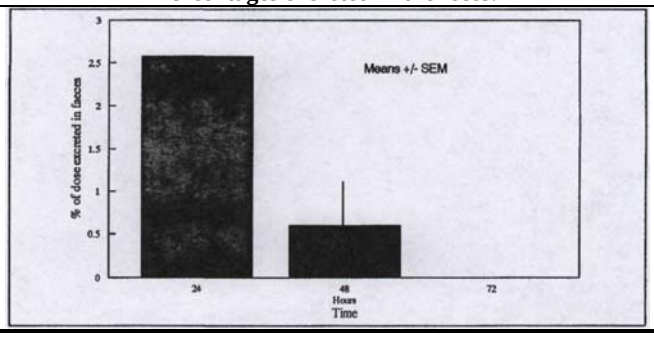
Data from Figures 5 and 6, p. 33 of 38, MRID 49009501.

Figure 5. Percentages of the dose of paraquat excreted in the urine and feces at various intervals following a single oral dose of 30 mg paraquat ion/kg bw.

Percentages excreted in the urine:



Percentages excreted in the feces:



Data from Figures 10 and 11, p. 37 of 38, MRID 49009501.

G. SACRIFICE AND PATHOLOGY:

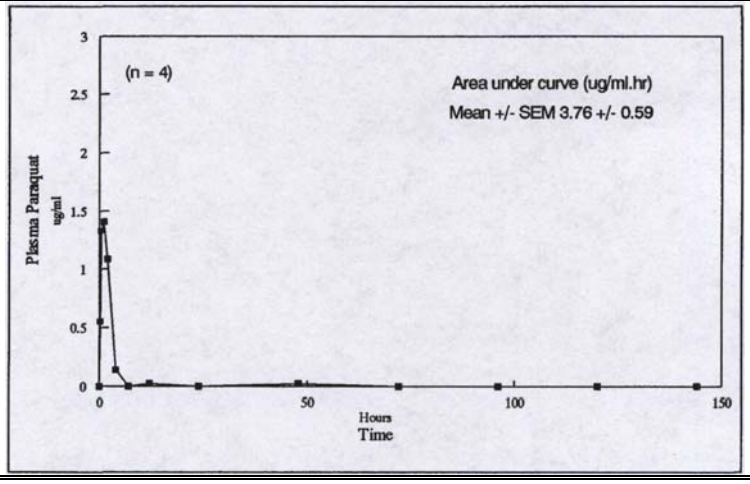
1. **Organ weight:** No findings reported

2. **Gross pathology:** No findings reported

3. **Microscopic pathology and tissue concentrations:** Figure 6 shows the concentrations of paraquat in blood plasma at various times after dosing with 2 mg paraquat ion/kg bw in Distribution and Excretion experiment XB2607. The concentration peaked at 1 hour and was below the limit of detection by about 7 hours, with the AUC being 3.76 ± 0.59 µg/mL.hr. Table 2 shows the concentrations of paraquat in the liver, lung, and kidney on day 7 following administration of that same dose. All concentrations were low. Paraquat levels in plasma were below the level of detection, which was 0.006 µg/mL. Table 3 shows the concentrations of paraquat in the blood plasma, liver, lung, and kidney at various intervals following administration of the 30 mg paraquat ion/kg bw dose, with these data coming from all three Distribution and Excretion experiments. Figure 7 shows a plot of the concentrations of paraquat in blood at that dose. The concentration of paraquat in plasma peaked about 1 hour after dosing, rapidly declined over the next 5 hours, and then more gradually declined up to 48 hours. However, the concentration of paraquat then increased substantially in the plasma by 72 hours. That increase was thought to result from reduced renal function. The AUC was 29.7 ± 3.4 µg/mL.hr for a total of 9 animals. Although there were increases (above the lowest level seen at 24 hours) in the concentration of paraquat in the kidneys and livers at 48 and 72 hours after treatment, no such increase was seen in the lungs. The concentration in the lungs at 72 hours was 54% of that seen at 1 hour, which was the time of the peak level in plasma.

In the Median Lethal Dose experiment, microscopic pathology of the kidneys in the animals with azotemia (*i.e.*, those showing accumulation of abnormally large amounts of nitrogenous waste products in the blood) showed multifocal hydropic change in the S_2 segment of the proximal tubules (see below). In the Distribution and Excretion experiments, microscopic pathological of the animals receiving the low dose revealed no treatment-related effects. However, at the dose of 30 mg paraquat ion/kg bw, the kidneys showed pronounced effects. At 24 hours, kidneys showed signs of multifocal hydropic change in the S_2 segment proximal tubule in 3 out of the 5 animals examined. At 48 hours, kidneys showed signs of multifocal hydropic change in the S_2 segment proximal tubule in all 5 animals examined, and in 2 of them there was interstitial fibrosis. At 72 hours, these kidney abnormalities had progressed, such that of the 4 animals examined, 2 had multifocal hydropic change in the S_2 segment proximal tubule, 3 had multifocal tubular necrosis in the S_2 segment proximal tubule, 4 had tubular dilation, 4 had luminal casts, 1 had interstitial nephritis, and 1 showed interstitial fibrosis. Histological examination of stomachs, duodenums, livers, and lungs of these same animals showed no compound-related effects, with the possible exception of one animal that, at 72 hours after dosing, had submucosal edema of the stomach wall, squamous metaplasia of the mucosa, and mucosa atrophy of the stomach. None of the 3 control animals showed any abnormalities in these organs in the microscopic pathology examination.

Figure 6. Concentrations of paraquat in plasma (in µg/mL) in rabbits at various intervals following administration of the 2 mg paraquat ion/kg bw dose in Distribution and Excretion experiment XB2607*.



Data from Figure 2, p. 30 of 38, MRID 49009501.

Table 2. Concentrations of paraquat in the liver, lung, and kidney of rabbits on day 7 following administration of the 2 mg paraquat ion/kg bw dose in Distribution and Excretion experiment XB2607.

	PARAQUAT (µg/g wet wt)		
	LIVER	LUNG	KIDNEY
MEANS	0.029	0.076	0.023
SD	0.031	0.029	0.007
SEM	0.016	0.015	0.004

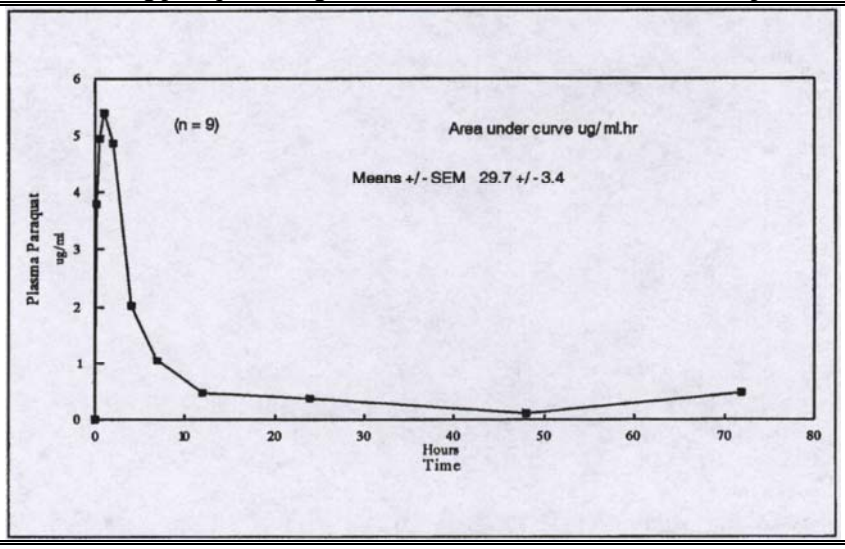
Data from Table 2, p. 27 of 38, MRID 49009501.

Table 3. Concentrations of paraquat in liver, lung, and kidney (in µg/g wet weight) and in plasma (in µg/mL) in rabbits at various intervals following administration of the 30 mg paraquat ion/kg bw dose in Distribution and Excretion experiments*.

TISSUE	1 HOUR	4 HOURS	24 HOURS	48 HOURS	72 HOURS
PLASMA	5.39 ± 0.22	2.01 ± 0.23	0.35 ± 0.13	0.11 ± 0.08	0.48 ± 0.14
KIDNEY	14.71 ± 4.80	3.03 ± 0.51	1.23 ± 0.144	2.48 ± 0.51	2.67 ± 0.50
LIVER	3.76 ± 1.10	2.16 ± 0.21	1.48 ± 0.10	1.75 ± 0.15	1.94 ± 0.24
LUNG	1.85 ± 0.91	1.48 ± 0.69	1.23 ± 0.16	1.12 ± 0.11	1.00 ± 0.21

Data from Table 3, p. 27 of 38, MRID 49009501.
*Means ± SEM, with at least 4 rabbits per time point.

Figure 7. Concentrations of paraquat in plasma (in µg/mL) in rabbits at various intervals following administration of the 30 mg paraquat ion/kg bw dose in Distribution and Excretion experiments.



Data from Figure 8, p. 35 of 38, MRID 49009501.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: The median lethal dose of paraquat in the female rabbit was between 40 and 50 mg paraquat ion/kg bw when administered by gavage. The peak concentration of paraquat in the blood plasma was reached within one hour after treatment, and the concentration in plasma returned fairly rapidly to zero or near zero levels. Only about 10% of the oral dose was absorbed, and it was excreted in the urine. At the oral dose of 30 mg paraquat ion/kg bw, there is both functional and morphological renal injury, which is thought to contribute to the mortality observed at higher doses. In contrast to some other mammalian species, paraquat at the oral dose of 30 mg paraquat ion/kg bw in the female rabbit does not accumulate in the lung to a significant extent or cause lung injury.

B. REVIEWER COMMENTS:

This non-guideline study provided useful information about the toxic effects of paraquat when administered orally as a single dose to female rabbits. The Reviewer agrees with the conclusions of the investigator. **The LOAEL is a single oral dose of 30 mg paraquat ion/kg bw based on renal damage revealed by azotemia and by microscopic pathology findings of multifocal hydropic change in the S_2 segment of the proximal tubules and additional renal damage. No NOAEL is indicated because of the limited testing of only 2 animals at all but one of the lower doses tested.** While no signs of toxicity were seen in any animals receiving oral doses as high as 12 mg paraquat ion/kg bw, some animals at the doses of 16, 20, and 24 mg paraquat ion/kg bw exhibited loss of appetite, and one animal at the 24 mg paraquat ion/kg bw dose had unspecified loss of weight and hematuria on day 8 of observation. The data are considered too sparse at the doses of 16, 20, and 24 mg paraquat ion/kg bw to provide the basis for a LOAEL.

C. STUDY DEFICIENCIES:

This study is non-guideline study. The study did not specifically mention that the treatment was by gavage. However, single oral doses were administered implying gavage adminstration. This slight deficiency had no impact on the reliability of the study.

APPENDIX

Details of the preparation and application of the oral doses used:

Dose preparations in the Median Lethal Dose experiment consisted of a mixture of unlabeled paraquat and deionized water such that a dose of 1 mL/kg bw was equivalent to a nominal dose per kg of animal body weight (Appendix Table 1). An additional lower dose group of 2 mg paraquat ion/kg bw was added to those shown in the table. In the Tissue Distribution and Excretion experiments, the two dose preparations consisted of a mixture of unlabeled and [^{14}C]-methyl labeled test substance formulated in the dose vehicle (deionized water) such that a dose of 1 mL/kg bw was equivalent to a nominal dose of [^{14}C]-paraquat ion (20 µCi) per kg bw.

A sample of each of the dose formulations used in the Distribution and Excretion experiments was analyzed prior to use to determine the achieved concentration of [^{14}C]-paraquat ion in water.

The levels of detection were set at 25 dpm above background, which resulted in limits of detection of about 2 and 25 ng for the experiments at 2 and 30 mg/kg bw, respectively.

Appendix Table 1. Percentages (v/v) of paraquat stock solution in the oral doses used in the Median Lethal Dose experiment.

Group	Percent of Y00061/160	Dose (mg Paraquat ion/kg)
1	1.047	4
2	2.094	8
3	3.14	12
4	4.188	16
5	5.236	20
6	6.283	24
7	7.85	30
8	10.47	40
9	13.02	50

Data from Table 1, p. 11 of 38, MRID 49009501.

DATA EVALUATION RECORD

PARAQUAT
STUDY TYPE: **Subchronic Neurotoxicity, feeding - mice**
NON-GUIDELINE
MRID 49122304

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 Crystal Drive
Arlington, VA 22202

Prepared by

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Task Order No.: 6-74

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Robert H. Ross, M.S. Program Manager

Signature: Robert H. Ross AE
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Quality Assurance:
Jennifer Goldberg, B.S.

Signature: Jennifer Goldberg AE
Date: 12/09/2013

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.
Summitec Corp. for the U.S. Environmental Protection Agency under Contract No. EP-W-11-014

EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** July 16, 2014
EPA Work Assignment Manager: Lori Brunsman **Signature:**
Science Info. Mgmt. Branch, Health Effects Division (7509P) **Date:** 7/17/14

Template version 09/11

TXR#: 0056764

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity, feeding - mice; (Non-guideline).

PC CODE: 061601 **DP BARCODE:** D412828

TEST MATERIAL (PURITY): Paraquat dichloride (99.9% a.i.) (Paraquat)

SYNONYMS: None provided

CITATION: Beck, M.J. (2013) Subchronic (91-day) dietary study to assess the effects of paraquat dichloride on dopaminergic neurons in C57BL/6J mice. WIL Research Laboratories, LLC, 1407 George Rd., Ashland, OH 44805-8946. Laboratory report number: WIL-639158, January 24, 2013. MRID 49122304. Unpublished. 1763 pages.

SPONSOR: Syngenta Crop Protection, LLC, 410 Swing Rd., PO Box 18300, Greensboro, NC 27419-8300.

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Hagerstown, MD 21742

EXECUTIVE SUMMARY:

In a nonguideline subchronic neurotoxicity study (MRID 49122304) paraquat dichloride (99.9% a.i., batch/lot # ASJ10083-03 [WIL ID no. 110018]) was administered continuously in the diet to 41 C57BL/6J mice/sex/group at dose levels of 0, 10 or 50 ppm (equivalent to 0, 1.7, and 10.2 mg/kg bw/day in males, respectively, and 0, 2.7, and 15.6 mg/kg bw/day in females, respectively) for 13 weeks. A positive control group of 31 C57BL/6J mice/sex was administered MPTP (l-methyl-4-phenyl-l,2,3,6-tetrahydropyridine hydrochloride), prepared in 0.9% physiological sterile saline, at 10 mg/kg bw/dose via 4 intraperitoneal (IP) injections spaced approximately 2 hours apart on a single day, 7 days prior to euthanasia, at a dose volume of 2.5 mL/kg/dose. Clinical examinations, body weights, and food consumption were recorded at selected intervals during the study. After 91, 92, 93, or 94 days of dietary exposure, 20 mice/sex/group designated for stereology assessments (Subset I) were anesthetized, perfused *in situ*, and the brains removed and shipped to Experimental Pathology Laboratories, Inc. for further processing and stereological analysis. After 4, 8 or 13 weeks of exposure, 5 mice/sex/time point from the 0, 10, or 50 ppm paraquat dichloride exposure groups and 5 mice/sex from the MPTP group at 13 weeks designated for pathological assessments (Subset II) were anesthetized, perfused *in situ*, and the brains removed, weighed and measured. The brains were shipped to NeuroScience Associates for sectioning and staining, and the slides were then shipped to Tox Path Specialists, LLC for neuropathological evaluation. After 95 days of exposure, 6 mice/sex/group designated for neurochemistry assessments (Subset III) were euthanized, the brain removed, and the striatum collected and shipped to RTI International for analysis of the concentrations of serotonin, dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA).

The analytical data indicated that the mixing procedure of the test diet was not adequate with % RSDs generally much greater than 10% (ranging from 8.9% to 68% RSD for the 10 ppm diet and 8.5% to 37% RSD for the 50 ppm diet). The variance between nominal and actual dosage to the animals was generally acceptable except for the 10 ppm diet formulations on the last sample day (150% of target); reanalysis revealed a concentration within 16% of target. Stability analysis revealed ending concentrations much greater than the starting concentrations. Although a previous validation study reported acceptable 12 day stability data, the results from this study were not acceptable.

Effects of treatment with paraquat dichloride were limited to transient, statistically significantly reduced body weight gain over the first week of treatment in males at 50 ppm (-83% of controls). Reductions in mean absolute body weight observed in males at 50 ppm were not biologically significant, being within 5% of the control values. Although the reduced body weight gain was transient, it was considered an adverse effect of treatment because it was not accompanied by a corresponding reduction in food consumption. No definitive, treatment-related effects on food consumption were observed during the study. Changes in mean absolute body weight and body weight gain in males at 10 ppm and females at 10 and 50 ppm were not considered biologically relevant. Treatment with 10 ppm or 50 ppm paraquat dichloride did not result in any observable clinical signs. Mortality of three males at 10 ppm and one male and one female at 50 ppm were not ascribed to treatment. No changes were noted during gross necropsy, and treatment with up to 50 ppm paraquat dichloride did not affect brain weight, brain measurements, stereological evaluation, neuropathological assessment of brain sections, or neurochemical concentrations of dopamine or its metabolites DOPAC and HVA in the striatal tissue of treated mice.

The response of the animals to the positive control was acceptable. Male and female mice treated with MPTP exhibited reversible clinical signs including hunched posture, piloerection, hypoactivity, and/or tremors following one or more dose administrations. These clinical signs were not present a week following dosing. In the week following dosing, males and females exhibited weight loss which was attributed to treatment with MPTP, but no definitive effects on food consumption were observed. Treatment with MPTP did not affect brain weights or measurements. A statistically significant decrease in tyrosine hydroxylase positive (TH^+) neurons and in the total contour volume was seen in male mice, but not female mice. In the striatal tissues from male mice treated with MPTP, marked decreases were observed in mean dopamine, DOPAC, and HVA concentrations with an associated increase in the mean dopamine turnover. The authors state that these findings are consistent with the known neurotoxicity of MPTP. The decreases in mean dopamine, DOPAC, and HVA concentrations observed for the striatal tissues obtained for the MPTP-treated female mice as compared to those for the control female mice were less than noted for the male mice.

Based on a transient decrease in body weight gain, the LOAEL was 50 ppm (10.2 mg/kg bw/day in males and 15.6 mg/kg bw/day in females), and the NOAEL was 10 ppm (1.7 mg/kg bw/day in males and 2.7 mg/kg bw/day in females).

The study is classified as **Unacceptable/ Non-Guideline**. The study is unacceptable because the analysis of the test diet formulations revealed unacceptable homogeneity and stability results. The study was not designed to meet guideline requirements.

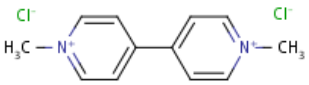
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Paraquat dichloride

Description:	coarse, light gray powder
Lot/Batch #:	ASJ10083-03 [WIL ID no. 110018]
Purity:	99.9% a.i.
Compound Stability:	Stable when refrigerated with desiccant, expiration date March 22, 2012
CAS # of TGAI:	1910-42-5
Structure:	



2. Vehicle: PMI Nutrition International, LLC Certified Rodent LabDiet® 5002

3. Positive control: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) (Batch # 090M1522V [WIL log no. 8739A]; 100% a.i., white powder) in 0.9% sodium chloride (Lot # C850073 and 05-402-DK)

3. Test animals:

Species:	Mice	
Strain:	C57BL/6J	
Age/weight at study initiation:	~10 wks; males: 18.0-26.8 g; females: 14.9-21.1 g	
Source:	The Jackson Laboratory, Bar Harbor, ME	
Housing:	Individually in clean, stainless steel, wire-mesh cages suspended above cageboard	
Diet:	PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*	
Water:	reverse-osmosis treated tap water *ad libitum*	
Environmental conditions:	**Temperature:**	21.2-22.9°C
	Humidity:	39.1-61.8%
	Air changes:	10 changes/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Minimum of 20 days	

B. STUDY DESIGN:

1. In life dates: Start: July 12, 2011; End: November 4, 2011

2. Animal assignment and treatment: Animals were randomly assigned to the test groups noted in Table 1 based on body weight stratification into a block design. Test substance was administered continuously in the diet for 91-95 days. The positive control substance, MPTP, prepared in 0.9% physiological sterile saline, was administered at 10 mg/kg bw/dose to mice via 4 intraperitoneal (IP) injections spaced approximately 2 hours apart on a single day, 7 days prior to euthanasia, at a dose volume of 2.5 mL/kg/dose.

Dose levels were chosen based on previous studies in mice. It was anticipated that dietary paraquat dichloride concentrations of 10 and 50 ppm would result in achieved dose levels of at least 1.2 and 6.0 mg paraquat ion/kg bw/day, respectively, over a 13-week period. These 2 dose levels were adequately spaced to allow the observation of any potential dose response, and the high-dose level of 50 ppm paraquat dichloride was expected not to exceed the maximum tolerated dose for mice of this strain (the author did not provide the maximum tolerated dose).

A summary of the study design is presented in Table 1. After 91, 92, 93, or 94 days of dietary exposure, 20 mice/sex/group designated for stereology assessments (Subset I) were anesthetized, perfused *in situ*, and the brains removed, processed, and subjected to stereological analysis. After 4, 8 or 13 weeks of exposure, 5 mice/sex/time point from the 0, 10, or 50 ppm paraquat dichloride exposure groups and 5 mice/sex from the MPTP group at 13 weeks designated for pathological assessments (Subset II) were anesthetized, perfused *in situ*, and the brains removed, weighed, measured, and evaluated for neuropathological changes. After 95 days of exposure, 6 mice/sex/group designated for neurochemistry assessments (Subset III) were euthanized, the brain removed, and the striatum collected and analyzed for the concentrations of serotonin, dopamine, DOPAC and HVA.

TABLE 1. Study design*

Experimental parameter		Dose group ppm (mg/kg bw/day)			Positive control
		Control (0 ppm)	Low dose (10 ppm)	High dose (50 ppm)	
Mean calculated test substance consumption (mg/kg bw/d)					
Expressed as Paraquat Dichloride	**Males**	-	2.4	14.1	-
	Females	-	3.7	21.5	-
Expressed as Paraquat Ion	**Males**	-	1.7	10.2	-
	Females	-	2.7	15.6	-
Total number of animals/sex/group		41/sex	41/sex	41/sex	31/sex
Stereological assessment (Subset I)		20/sex	20/sex	20/sex	20/sex
Pathological evaluation (Subset II)		15/sex	15/sex	15/sex	5/sex
Neurochemistry assessment (Subset III)		6/sex	6/sex	6/sex	6/sex

Data from page 25 of study report MRID 49122304

3. Test Substance preparation and analysis:

Diet was prepared approximately weekly. For the control group (Group 1) and the positive control group (Group IV), an appropriate amount of basal diet (PMI Nutrition International, LLC Certified Rodent LabDiet®) was weighed, placed in a labeled storage bag, and stored at room temperature. In the paraquat dichloride treatment groups, paraquat dichloride was dried in an oven at approximately 100ºC for a minimum of 4 hours. The Group 3 diet was prepared by adding the test substance to the diet and mixing. A portion of the Group 3 test diet was then diluted with additional basal diet to obtain the Group 2 dietary formulation.

Prior to the initiation test diet administration (July 16, 20, 26, 28), test diets were prepared at concentrations of 10 and 50 ppm paraquat dichloride, and samples were collected from the top, middle, and bottom of the 10 and 50 ppm test diet formulations and analyzed for homogeneity. The samples (top, middle, and bottom) were then combined and stored for 12

days at room temperature for stability determinations. Samples were also collected from the top, middle, and bottom of each test diet and the middle of each control diet used on the study during the first (study week 0; July 28), fourth (study week 3; August 18), eighth (study week 7; September 15 and 19), and thirteenth (study week 12; October 20) weeks of treatment. Due to unacceptable results for the first 10 ppm test diet preparation for Group 2 (study week 0, July 28), the 10 ppm diet was remixed the day after formulation; samples for homogeneity were collected, combined, and stored for 12 days at room temperature for stability determinations. All analyses were conducted using HPLC with mass spectrometry detection.

Additionally, in a previous method validation study, homogeneity and a 12-day room temperature stability of paraquat dichloride at dietary concentrations of 25 and 100 ppm were established (WIL-639105).

The positive control substance, MPTP, was prepared by weighing an appropriate amount of MPTP into a sterile glass septum vial, adding the appropriate amount of the vehicle (0.9% physiological sterile saline), capping with a sterile septum, and swirling/inverting until the MPTP was dissolved. The formulation was then filtered using a 0.22 µm PVDF syringe filter. Formulations were prepared within 5 days of use, divided into aliquots for dispensation, and stored refrigerated. A sample for concentration verification was collected and analyzed using HPLC with u.v. detection. Additionally, in a previous study, 6-day refrigerated stability and homogeneity of MPTP formulations at 4 mg/mL were established.

Results

Homogeneity analysis: The homogeneity analysis of the 10 and 50 ppm diet prior to the initiation test diet administration revealed the following:

10 ppm:

July 16: mean concentration 57.9% of target concentration, %RSD of 43%
Reanalysis on July 18: mean concentration 89.4% of target, %RSD of 68%
July 20: mean concentration 99.6% of target concentration, %RSD of 24%
July 26: mean concentration 97.8% of target concentration, %RSD of 24%
July 28: mean concentration 86.4% of target concentration, %RSD of 22%
Reanalysis on August 1: 96.4% of target concentration, %RSD of 8.9%

50 ppm:

July 20: mean concentration 81.1% of target concentration, %RSD of 26%
July 26: mean concentration 110% of target concentration, %RSD of 37%
July 28: mean concentration 95.3% of target concentration, %RSD of 8.5%

Stability analysis: The stability analysis of the 10 and 50 ppm test diet formulations after 12 days at room temperature revealed mean concentrations that were 100% and 155%, respectively, of initial concentrations.

Concentration analysis: The mean concentration analyses of the 10 and 50 ppm diet revealed the following:

10 ppm:

July 28: mean concentration 86.4% of target concentration, %RSD of 22%

Reanalysis on August 1: 96.4% of target concentration, %RSD of 8.9%
August 18: mean concentration 104% of target concentration, %RSD of 20%
September 15: mean concentration 101% of target concentration, %RSD of 14%
September 19: mean concentration 93.5% of target concentration, %RSD of 11%
October 20: mean concentration 150% of target concentration, %RSD of 32%
Reprocessed; analysis November 1: 116% of target concentration, %RSD of 21%

50 ppm:
July 28: mean concentration 95.3% of target concentration, %RSD of 8.5%
August 18: mean concentration 94.2% of target concentration, %RSD of 18%
September 15: mean concentration 95.3% of target concentration, %RSD of 7.4%
September 19: mean concentration 91.5% of target concentration, %RSD of 3.2%
October 20: mean concentration 87.6% of target concentration, %RSD of 40%
Reprocessed; analysis November 1: 105% of target concentration, %RSD of 50%

The mean concentration of the MPTP positive control formulation was 103% of the target concentration.

The analytical data indicated that the mixing procedure was not adequate with % RSDs generally much greater than 10%. The variance between nominal and actual dosage to the animals was generally acceptable except for the 10 ppm diet formulations on the last sample day; reanalysis revealed a concentration within 16% of target. Stability analysis revealed ending concentrations much greater than the starting concentrations. Although a previous validation study reported acceptable 12 day stability data, the results from this study were not acceptable.

4. **Statistics:** Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean, and standard error (S.E.) was additionally presented for body weight, food consumption, and brain weight and measurement data.

With the exception of the analysis of the stereology data, all statistical tests were performed using WTDMS™. Analyses were conducted using 2-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the control group. The positive control substance-treated group was compared separately to the control group.

Body weight, body weight change, food consumption, and brain weight/measurement data were subjected to a parametric one way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test or a two-sample t-test, as appropriate, was used to compare the test substance- and positive control substance-treated groups to the control group. The reviewer notes that the study authors do not state whether or not homogeneity of variance was first determined to assess whether parametric or nonparametric analyses should be conducted. The reviewer decided not to do a re-analysis of the data on the basis that the changes in values were generally not biologically significant.

The effects of treatment on the concentration of neurotransmitters (dopamine, 5HT) and dopamine metabolites (DOPAC, HVA) and the estimate of dopamine turnover as derived from neurochemistry analyses performed by RTI was evaluated for homogeneity of variance

using the Bartlett's test. If the Bartlett's test was not statistically significant, then an ANOVA was conducted as described above. If transformation of the data (e.g., logarithmic, square root, inverse) does not result in the assumption of homogeneity being met (i.e., a non-significant Bartlett's test), then a non-parametric analysis (Mann-Whitney-Wilcoxon Test) was conducted.

Statistical analyses of the stereology data were conducted by Sielken & Associates Consulting, Inc. The effects of treatment (PQ or MPTP) on the mean number of neurons in the substantia nigra pars compacta (SNpc), as determined by stereological analysis, was assessed for the following parameters using a one-sided t-test: dopaminergic (TH^+) neurons, total ($TH^+ + TH^-$) neurons, and contour volume. Differences in means were considered statistically significant at $p \leq 0.05$.

Group mean scores based on the categorical classification of microscopic findings were calculated by Tox Path Specialists, LLC but statistical analyses were not conducted.

C. ANTEMORTEM METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed twice daily for mortality and moribundity. Detailed clinical observations were recorded weekly. Animals administered MPTP were also observed for signs of toxicity at approximately 1 hour following each MPTP administration.

2. **Body weight:** Animals were weighed weekly, beginning 2 weeks prior to test diet administration. Mean body weight changes were calculated for study days 0-28, 0-56, and 0-91.

3. **Food consumption:** Individual food consumption was recorded weekly, beginning 2 weeks prior to test diet administration. Food intake was calculated as g/animal/day for the corresponding body weight intervals. Mean test substance consumption (mg/kg bw/day) for each group was determined by multiplying the concentration of the test substance in the diet (mg/kg of diet) by the g/animal/day food consumption value and dividing by the body weight (in kg) for each interval.

D. POSTMORTEM METHODS / OBSERVATIONS:

1. **Unscheduled deaths:** A complete necropsy was conducted on all animals found dead or euthanized *in extremis*; moribund animals were euthanized by cervical dislocation. The necropsy included examination of the cranial, thoracic, abdominal, and pelvic cavities. The kidneys, lungs, and any gross lesions were retained in 10% neutral-buffered formalin for possible future histopathological examination. The carcasses were then discarded.

2. Scheduled euthanasia:

a. Stereology (Subset I): After 91, 92, 93, or 94 days of exposure, 20 mice/sex/group were anesthetized with sodium pentobarbital and perfused *in situ*. The mice were flushed with chilled 0.9% physiological saline, followed by 4% paraformaldehyde (pH approximately 9.5); the flow rate was approximately 10 mL/minute. Following removal of the head, the brain (including olfactory bulbs) was removed from the skull by 1 prosector. The perfusion-fixed brain was removed from the skull and preserved in 4% paraformaldehyde. The instruments used to remove the brain were rinsed with saline between animals. Following collection of the brains, the lungs and kidneys of mice in Groups 1-3 were collected and preserved in 10% neutral-buffered formalin for possible future histopathologic examination. The carcasses were then discarded.

The whole brains were placed in a container with cool packs and shipped overnight to Experimental Pathology Laboratories, Inc. There, the midbrain region was isolated from each brain and subjected to immersion-fixation by placing the samples in a 50-mL conical centrifuge tube containing approximately 40 mL of 4% paraformaldehyde solution; the samples were allowed to immersion-fix for approximately 24 hours at 4°C. After a minimum of 24 hours, the midbrain samples were transferred to 10% sucrose solution and stored at approximately 4°C. After another 24 hours, the midbrains were transferred to 30% sucrose solution and stored at approximately 4°C for a minimum of 48 hours. The midbrains were then flash frozen in isopentane (-40°C) for approximately 35 seconds. Each midbrain was then wrapped in aluminum foil, appropriately labeled, and stored at ≤-70°C until processed for stereological analysis.

Final processing and the stereology assessments were performed at Experimental Pathology Laboratories, Inc. (EPL). Just prior to microtomy, each brain sample was removed from the freezer, and then serially microtomed in the coronal plane at 40 micron. Microtomed sections were placed in wells containing an antifreeze solution (30% ethylene glycol) at -20°C, which also served as a temporary storage medium. The matrix grid pattern of the wells allowed every third section that contained the SNpc (Substantia Nigra Pars Campacta) to be selected for immunohistochemical staining and stereological analysis.

To maximize antibody penetration by allowing the antibodies access to both the top and bottom of each section, brain sections were suspended free-floating in mesh wells during the immunostaining procedures. Immunostaining was performed according to the standard avidin-biotin complex (ABC) method.

Stereology assessment provided an unbiased estimate of the number of tyrosine hydroxylase (TH) positive (TH^+), TH negative (TH^-) neurons, and of the total number of neurons (TH^+ plus TH^-) in the SNpc in the mice. The total number of TH^+ and TH^- neurons in the SNpc was estimated using the optical fractionator approach which employed an unbiased systematic random sampling methodology. Accordingly, neuron cell bodies were counted in a subsample of sections, section thicknesses, and section areas, and then the results were extrapolated to provide estimates of total number of TH^+ and TH^- neurons in the left and right SNpc. The evaluation was performed blindly, with the operator unaware of the treatment group status of individual mice, and the order of animal evaluation followed a block-randomized design.

Neuronal cell counts for each animal, and other relevant details of the stereological analysis (e.g., Z-depth area, estimates of the total area and volume of the SNpc), were exported to an individual Excel spreadsheet file. Additionally, the Stereo Investigator software generated and exported coefficient of error (CE) values for each of the TH^+ and TH^- neuron counts as guidelines for determining whether the stereological sampling was sufficiently extensive (CE < 0.1). To assess intra-study variability, chromogenic-stained sections from two control animals were evaluated periodically and blindly within the randomized block order of the stereological assessment; thus, these animals served as internal controls. Control group male No. 19798 was counted six times, and control group female No. 19553 was counted four times. For each of these mice, only the first count was used to assess potential treatment effects.

b. Pathology: Detailed Neuropathology of the Substantia Nigra Pars Campacta (SNpc) and Striatum (Subset II): Five mice/sex/group (Groups 1-3) were euthanized after 31, 59, or 94 days of exposure, and 5 mice/sex in Group 4 were euthanized during study week 13 (7 days after MPTP treatment). Mice were anesthetized by intraperitoneal injection of sodium pentobarbital and perfused *in situ*. The mice were flushed with approximately 25 mL of sodium cacodylate buffer, followed by perfusion with approximately 75 mL of sodium cacodylate-based 4% paraformaldehyde (methanol free). Following perfusion, the head was removed. The skin on the head was removed all the way down to the snout, and the color of the dorsal surface of the skull was documented. Following the skinning process, the head (with calvaria intact) was placed in cacodylate-based 4% paraformaldehyde for approximately 23 hours. The brain (including olfactory bulbs) was then removed from the skull and weighed, and the size (length [without the olfactory bulbs] and width) was recorded. Any abnormal coloration or lesions of the brain or spinal cord were recorded. The intact brains were placed into sodium cacodylate buffer solution and maintained refrigerated for a minimum of 24 hours. The brain samples, fully immersed in cacodylate buffer, were placed in a container, maintained at approximately 4°C, and transported to Neuroscience Associates, Inc. for processing. In addition, the lungs and kidneys of mice in Groups 1-4 were collected and preserved in 10% neutral-buffered formalin for possible future histopathologic examination. The carcasses were then discarded.

At Neuroscience Associates, Inc., the brains were trimmed to isolate the area of the substantia nigra to the striatum. All brains were then multi-embedded and sectioned at 30 µm in the coronal plane through the striatum to substantia nigra. Serial sections were produced and stained as described in the following table. Although these various histologic processing techniques are referred to as "stains", several of the immunological techniques used to characterize the various neuronal/glial properties are not actually stains per se:

Stain	Measurement
TH (Tyrosine hydroxylase)	Detection of dopaminergic neurons and their neuronal processes, including synaptic terminals in the striatum
GFAP (Glial fibrillary acidic protein)	Astrocyte reactions
IBA-1 (Ionized Calcium Binding Adaptor Molecule 1)	Microglial reactions
AmCuAg (Amino Cupric Silver)	Neuronal necrosis
Caspase 3	Detection of apoptosis; only SNpc sections were evaluated for caspase 3
Thionine	General morphology
Micro ApoTag (TUNEL) (Terminal deoxynucleotidyl transferase dUTP nick end)	Detection of apoptosis; method for detecting DNA fragmentation, only sections of the SNpc were processed for TUNEL

All slides from the region of the substantia nigra were shipped to Tox Path Specialists, LLC for examination. Data were recorded according to a semi-quantitative scoring system, including analysis of the presence of necrotic cells, the character of any glial reaction, an assessment of the degree of dopaminergic neuron loss (if any), and an assessment of the possible mechanism for the loss. In accordance with the protocol, the following were assessed in addition to other findings:

- Presence of necrotic cells: This was performed primarily by examination of the AmCuAg-stained sections. Apoptosis as an explanation of cell death was assessed using the Caspase-3 and TUNEL stains.
- Character of the glial reaction: The glial reaction was assessed during examination of the GFAP and IB-A-1-stained slides.
- Degree of dopaminergic neuron loss (if any): This was assessed using the AmCuAg stain for general cell necrosis/death and the TH stain. A decrease of TH staining of neurons in the pars compacta of the substantia nigra would indicate either cell loss (verified with the AmCuAg stain) or a loss of tyrosine expression in the neuronal population. A decrease of TH staining in the striatum would indicate a loss (absolute or functional) of dopaminergic neurons as expressed by a decreased amount of TH in the synaptic terminals in the striatum.
- Assessment of the possible mechanisms for neuronal loss (if present): This was conducted using the Caspase-3 and TUNEL stains.

c. Neurochemistry: Striatal Dopamine and Dopamine Metabolite Concentration Analysis (Subset III): After 95 days of exposure, 6 mice/sex/group were euthanized via cervical dislocation followed by decapitation with scissors. Brain samples were collected by 2 prosectors. In order to minimize cross contamination, each prosector had access to 3 stations (1 for the control group, 1 for the test substance-treated groups, and 1 for the positive control substance group, as necessary) with separate instruments at each station. Instruments were rinsed with saline between animals. In addition, the number of animals from each group was approximately equally distributed between the 2 prosectors. Lastly and when possible, animals were euthanized in a counterbalanced random block design with up to 6 blocks (1 mouse/group/block, if possible) such that the mice within each block were euthanized in random order.

Immediately after euthanasia, the brain of the animal was rapidly removed, placed in a mouse brain matrix, and the appropriate coronal sections were prepared. The coronal section containing the striatum was placed on a cold surface. The left and right striatum were dissected free, weighed together, and placed in an appropriately labeled vial. All striatal samples were immediately flash frozen in liquid nitrogen after collection and stored frozen (approximately -70°C) until shipped for analysis. The midbrain (caudal to the razor cut and including the substantia nigra) was collected, flash-frozen in liquid nitrogen, and stored frozen (approximately -70°C). The remaining portion of the brain (after the striatum and midbrain were collected) was discarded. The left and right hemisphere striatum samples were shipped on dry ice via overnight carrier to RTI International for neurotransmitter analysis by electrochemical detection. At RTI International, striatal brain samples were analyzed using high pressure liquid chromatography (HPLC) coupled with electrochemical detection (ECD) to quantitate dopamine and the two dopamine metabolites HVA and DOPAC. Although serotonin (5-hydroxytryptamine; 5-HT) concentrations were also supposed to be quantified, the HPLC-EC method for quantifying 5HT did not pass earlier validation experiments. Therefore, differences in striatal serotonin concentration were not evaluated in this study.

II. RESULTS:

A. ANTEMORTEM OBSERVATIONS:

1. **Clinical signs:** No treatment-related clinical signs were observed. Clinical signs occurred in low incidence, in a non-dose related matter, were sporadic, or occurred in similar incidence in the control group.

   Clinical signs in males administered MPTP included hunched posture, hypoactivity, and/or tremors with increasing incidence at the time of the second dose administration and 1 hour following the second, third, and fourth doses (up to 13 males following the fourth dose). Piloerection was observed for 1 male at the time of the second dose administration, 1 male at 1 hour following the second dose administration, and another male at the time of the third dose administration.

   Clinical signs in females administered MPTP included hunched posture, piloerection, and hypoactivity, and were observed for 2-7 females 1 hour following the second, third, and/or fourth doses. One hour following the fourth dose, tremors were also observed for a single female. None of the behavior-related findings observed for males and females in the MPTP group persisted to the weekly clinical examination that was conducted 1 week after dose administration.

2. **Mortality:** Three males from the 10 ppm group were found dead (one each on study day 5, 14, and 42), and one male from the 50 ppm group was found dead on study day 84. No significant clinical signs or gross necropsy findings were observed; therefore, the cause of death could not be determined. Based on the lack of dose response of the mortalities, they were not considered to be related to treatment.

One female from the 50 ppm group was euthanized *in extremis* on study day 77. Clinical signs observed in this female included hunched posture and decreased defecation on the day of euthanasia, and weight loss the week prior to euthanasia. Gross necropsy did not reveal any abnormalities; therefore, the moribundity was not considered an effect of treatment.

3. **Body weight and body weight gain:** In the mice treated with paraquat dichloride, mean absolute body weight was statistically significantly reduced in males at 50 ppm on days 35, 42, 56, and 77 (↓4.3, 4.3, 3.5, and 4.1% of controls, respectively). No statistically significant differences in mean absolute body weights were noted in males at 10 ppm or females at 10 or 50 ppm (Table 3).

   Mean body weight gain was statistically significantly reduced in males at 50 ppm over days 0-7 (↓83%) and was considered related to treatment. Other statistically significant differences noted in weekly mean body weight changes in male and female mice treated with 10 or 50 ppm paraquat dichloride were not considered treatment related because they did not follow a clearly defined dose response and included both increases and decreases in weight compared to control values.

   Males and females in the positive control group exhibited body weight loss in the week following treatment (days 84-91) with MPTP (males: -0.4 g vs. -0.1 for controls; females -0.1 g vs. 0.4 g for controls); this weight loss was considered an effect of the treatment.

   Selected mean absolute body weight and body weight gain data are summarized in Table 3.

TABLE 3. Body weight and body weight gain [a, b, c]

Observation (g ± s.d.)	Dose level paraquat dichloride			
	Control	10 ppm	50 ppm	MPTP
Body weight-Males				
Day 0	22.6 ± 1.8	22.5 ± 1.7	22.4 ± 1.6	22.2 ± 1.9
Day 42	25.6 ± 1.6	25.5 ± 1.7	24.5** ± 1.4 (↓4.3%) [d]	25.1 ± 1.7
Day 91	27.1 ± 1.5	27.3 ± 1.5	26.4 ± 1.4	26.5 ± 1.3
Body weight-Females				
Day 0	17.2 ± 1.0	17.0 ± 1.0	17.1 ± 0.9	17.1 ± 1.2
Day 42	20.4 ± 1.3	20.0 ± 1.7	20.2 ± 1.4	20.3 ± 1.6
Day 91	22.2 ± 1.2	21.7 ± 1.4	22.3 ± 1.0	22.0 ± 1.4
Body weight gain-Males				
Day 0-7	0.6 ± 0.6	0.6 ± 0.5	0.1** ± 0.5 (↓83%)	0.6 ± 0.5
Day 0-28	1.8 ± 0.9	2.0 ± 0.9	1.5 ± 1.0 (↓17%)	2.1 ± 0.86
Day 0-56	3.3 ± 1.3	3.8 ± 0.9	2.7*± 1.0 (↓18%)	3.4 ± 1.1
Day 0-91	4.3 ± 1.3	4.6 ± 1.0	3.8 ± 1.2 (↓12%)	4.3 ± 1.1
Body weight gain-Females				
Day 0-7	0.3 ± 0.5	0.4 ± 0.5	0.3 ± 0.6	0.3 ± 0.6
Day 0-28	2.1 ± 1.2	2.2 ± 1.1	2.0 ± 1.3	2.1 ± 1.1
Day 0-56	3.6 ± 1.1	3.5 ± 1.2	3.4 ± 1.1	3.9 ± 0.9
Day 0-91	4.8 ± 0.9	4.8 ± 0.9	5.2 ± 1.1	4.9 ± 0.9

[a] Data were extracted from MRID 49122304, pp. 51-66.
[b] Values represent mean ± SD
[c] N = 31 animals in the MPTP male and female groups; in males and females treated with 0, 10,or 50 ppm paraquat dichloride, n ranged from 30-41
[d] Numbers in parenthesis are the percentage difference compared to controls; calculated by reviewer except for value on Day 43 (which was calculated by study author).
*=p<.05, **=p<.01, when compared to control means.

4. **Food consumption**: Treatment of mice with paraquat dichloride did not adversely affect food consumption. The statistically significant differences noted in food consumption in male and female mice fed both 10 ppm or 50 ppm were not considered an effect of treatment because they were not related to dose, and were noted prior to the introduction of the test material (study week -7 to 0).

   In mice treated with MPTP, food consumption was statistically significantly decreased in males during the week following treatment (5.3 g vs. 6.2 for controls), but this reduction was similar to that seen the previous week before treatment (5.8 g vs. 7.4 g for controls). Food consumption in females was not statistically significantly different from controls in the week following treatment.

   Test substance intake (mg/kg bw/day) was calculated based on body weight gain data and food consumption data, and is summarized in Table 1.

B. POSTMORTEM OBSERVATIONS:

1. **Gross necropsy (scheduled euthanasia; Subset I and Subset II):** No gross necropsy changes related to treatment with paraquat dichloride were observed.

   In the stereology group (Subset I), changes not related to treatment included one male at 50 ppm with changes in the kidneys (small; dilated renal pelvis) and ureters (distended).

   In the pathology group (Subset II), findings of red areas and/or red discoloration of the brain were found in both treated and control mice, and are therefore not considered an adverse effect of treatment. These data are summarized in Table 4.

TABLE 4. Macroscopic findings of Mice in the Pathology Group (Subset II) [a, b]				
Observation (g ± s.d.)	Dose level paraquat dichloride			
	Control	10 ppm	50 ppm	MPTP
Males				
Week 4: Brain (n) [c]	5	5	5	0
Area(s) of red	1	1	2	
Discoloration, red	1	4	4	
No significant changes	4	1	1	
Week 8: Brain (n)	5	3	5	0
No significant changes	5	3	5	
Week 13: Brain (n)	5	5	5	5
Area(s) of red	2	2	2	1
Discoloration, red	0	1	2	2
No significant changes	3	3	3	3
Females				
Week 4: Brain (n)	5	5	5	0
Area(s) of red	2	3	2	
Discoloration, red	2	2	4	
No significant changes	1	1	0	
Week 8: Brain (n)	5	5	5	0
No significant changes	5	5	5	
Week 13: Brain (n)	5	5	5	5
Area(s) of red	1	1	1	0
Discoloration, red	1	0	1	1
No significant changes	4	4	3	4

[a] Data were extracted from MRID 49122304, pp. 83-88.
[b] Values represent number of animals
[c] Number of animals examined

2. **Brain weights and measurements (Subset II and Subset III):** In mice from Subset II, no statistically significant or biologically significant differences in final body weight, brain weight, brain length, or brain width measurements were noted in male or female mice fed 10 ppm or 50 ppm paraquat dichloride when assessed at 4, 8, or 13 weeks of treatment, or in male or female mice in the positive control group at 13 weeks.

   In mice from Subset III, no statistically significant or biologically significant differences in the brain striatum weight were noted in male or female mice fed 10 ppm or 50 ppm paraquat dichloride when assessed at 13 weeks of treatment, or in male or female mice in the positive control group at 13 weeks.

3. **Stereology assessment (Subset I):**

   No effects of paraquat dichloride treatment were evident when evaluating the number of TH^+, TH^-, or the total amount of neurons in the SNpc of male or female mice fed 10 or 50 ppm paraquat dichloride. The total contour values for the paraquat dichloride treated mice were comparable to those of the controls.

   In the MPTP positive control group, a statistically significant decrease in TH^+ neurons and in the total contour volume was seen in the male mice, while no statistically significant differences were observed in female mice in the positive control group.

   Stereology data are summarized in Table 5 and Figure 1.

Table 5. Stereological Findings in the Substantia Nigra Pars Compacta (SNpc)

Treatment	Statistical Parameter	Males				Females			
		TH^+ Neurons	TH^- Neurons	Total Neurons (TH^+ & TH^-)	Total Contour Volume (μm^3)	TH^+ Neurons	TH^- Neurons	Total Neurons (TH^+ & TH^-)	Total Contour Volume (μm^3)
Control (Group 1)	n	20				20			
	mean	15,173	7,510	22,683	345,661,900	14,813	6,024	20,838	339,634,150
	sd [a]	2,447	2,039	3,197	27,303,337	2,657	1,442	2,937	29,853,427
MPTP 4x10 mg/kg (Group 4)	n	17[b]				20			
	mean	13,675	7,599	21,274	313,410,412	14,004	6,298	20,302	329,448,500
	sd	2,298	1,816	3,499	32,778,538	2,344	1,420	2,692	34,881,823
	% change	-10	1	-6	-9	-5	4	-3	-3
	t-test p-value	**0.0317***	0.4444	0.1063	**0.0015****	0.1568	0.2747	0.2756	0.1643
Paraquat 10 ppm (Group 2)	n	20				20			
	mean	15,624	7,618	23,241	337,552,950	15,482	6,357	21,840	340,888,400
	sd	2,208	1,751	2,344	29,747,913	2,416	1,268	2,788	25,223,816
	% change	3	1	2	-2	4	5	5	0
	t-test p-value	0.2724	0.4294	0.2664	0.1874	0.2049	0.2218	0.1377	0.4433
Paraquat 50 ppm (Group 3)	n	19[c]				18[d]			
	mean	14,794	7,291	22,085	333,401,158	15,128	6,086	21,214	331,550,056
	sd	2,241	1,971	3,245	20,940,608	2,594	1,305	2,910	33,356,215
	% change	-2	-3	-3	-4	2	1	2	-2
	t-test p-value	0.3082	0.3677	0.2829	0.0615	0.3569	0.4456	0.3470	0.2191

[a] SD = standard deviation; *Significant $p \leq 0.05$; **Significant $p \leq 0.01$

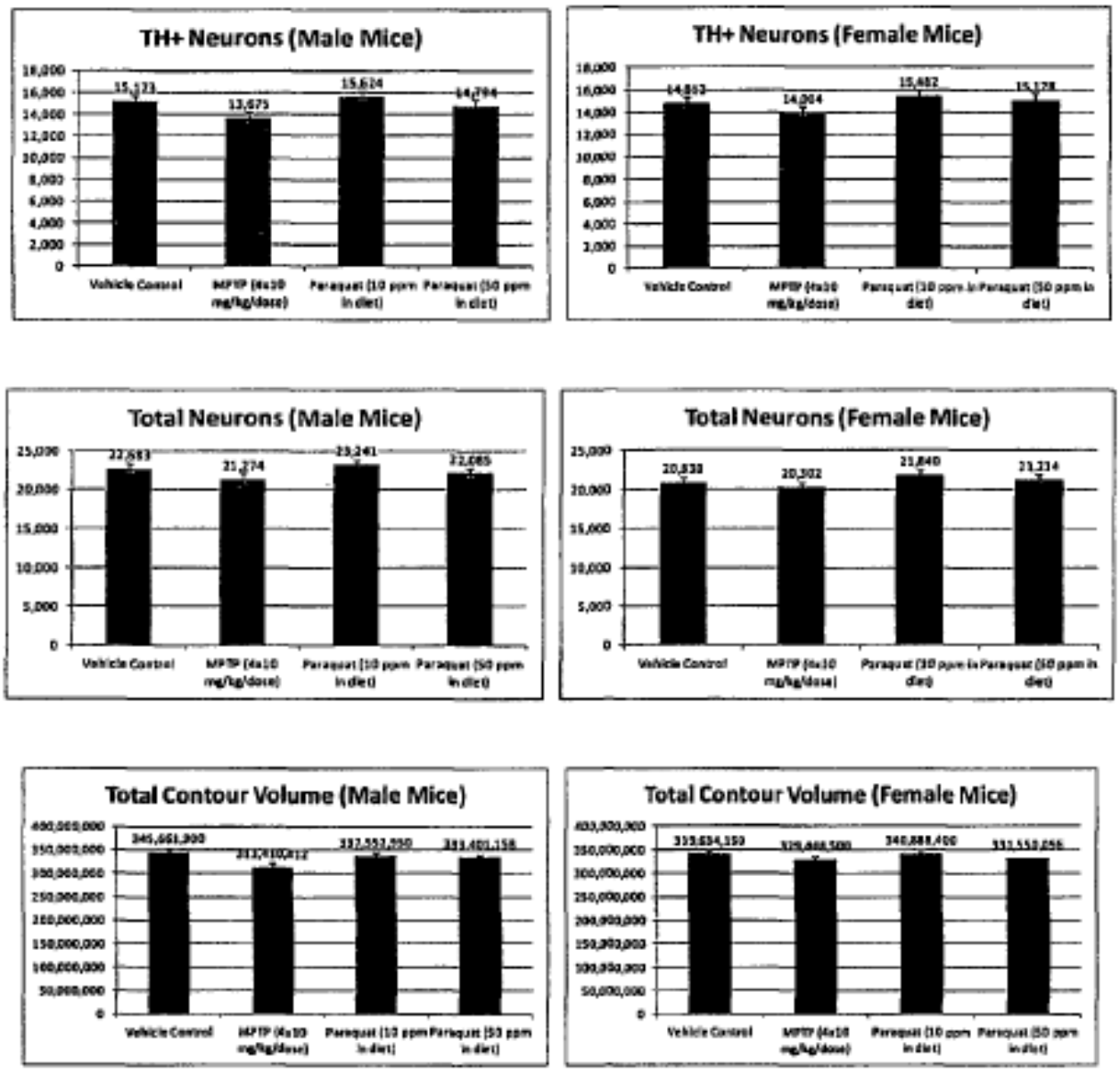
[b] Three mice from this group could not be evaluated due to irreparable tissue artifacts

[c] One mouse from this group could not be evaluated due to irreparable section artifacts

[d] Two mice from this group could not be evaluated due to irreparable section artifacts

Table reproduced from MRID 49122304, p. 219.

FIGURE 1: STEREOLOGICAL FINDINGS IN THE SUBSTANTIA NIGRA PARS COMPACTA



Error bars are plus and minus one standard error of the mean.
Reproduced from MRID 49122304, p. 220

b. Pathology: Detailed Neuropathology of the Substantia Nigra Pars Campacta and Striatum (Subset II): No treatment-related changes in the microscopic pathology of the examined brain sections were observed from mice fed 10 ppm or 50 ppm paraquat dichloride. Specifically, no neuronal or glial changes were noted in the substantia nigra or the striatum.

It is noted that none of the gross lesions noted during macroscopic examination of the paraquat dichloride treated mice (Table 4, red areas of the brain and/or red discoloration of the brain) were accompanied by microscopic changes. The author remarked that

discolorations and/or red areas are very common in the brain of animals, especially those that are perfusion fixed, because of slight variations in the pooling of blood or the degree to which regional blood vessels were voided of red blood cells (in perfusion fixed animals).

In contrast, pathological changes were observed in the brains of mice treated with MPTP.

- Tyrosine hydroxylase (TH) stain
  - When viewing the striatum tissues stained with the TH stain, 5/5 males and 5/5 females had decreased TH staining which was due to a loss of synaptic terminals from dopaminergic neurons in the pars compacta region of the substantia nigra.
  - In the Substantia Nigra Pars Compacta, 3/5 males but 0/5 females exhibited decreased TH Staining/Decreased TH+ Neurons.
  - In the Ventral Tegmental Area, decreased TH+ Neurons was observed in 3/5 males but could not be detected in the females.
- Glial Fibrillary Acidic Protein (GFAP) Staining
  - When viewing the striatum tissues stained with the GFAP stain, 5/5 males and 5/5 females had increased staining which was due to an increase in reactive astrocytes responding to the injury to synaptic terminals in the region
  - In the Substantia Nigra Pars Compacta, 1/5 males but 0/5 females exhibited an increase in the GFAP staining due to an increase in the reactive astrocytes responding to the injury to dopaminergic neurons in the region
- Ionized Calcium Binding Adapter Molecule - 1 (IBA-1) Staining
  - When viewing the striatum tissues stained with the IBA-1 stain, 5/5 males and 5/5 females had increased staining
  - In the Substantia Nigra Pars Compacta, 1/5 males but 0/5 females exhibited an increase in the IBA-1 staining

c. Neurochemistry: Striatal Dopamine and Dopamine Metabolite Concentration Analysis:

When evaluating the neurochemical concentrations in the striatal tissue of mice fed 10 or 50 ppm paraquat dichloride, no treatment-related differences were observed in the concentrations of dopamine, DOPAC, or HVA or in the dopamine turnover rate. The statistically significantly reduced HVA concentration in females at 10 ppm was not considered an adverse effect of treatment because it was not dose related.

In the striatal tissues from male mice treated with MPTP, marked decreases were observed in mean dopamine, DOPAC, and HVA concentrations compared to those for the control male mice. An associated increase in the mean dopamine turnover, relative to control, was also determined for striata obtained from the MPTP-treated male mice. The authors state that these findings are consistent with the known neurotoxicity of MPTP. The decreases in mean dopamine, DOPAC, and HVA concentrations observed for the striatal tissues obtained for the MPTP-treated female mice as compared to those for the control female mice were less than noted for the male mice. The study authors note: "MPTP has previously been demonstrated to induce greater neurotoxicity in male mice than in female mice as evidenced by greater striatal dopamine depletions. Although the relative decrease in DOPAC concentration noted in the MPTP-treatment female mice was somewhat similar to that observed in male mice, the extent of this decrease was likely a reflection of the variability of DOPAC concentrations in the control female striatal samples (i.e., the mean DOPAC concentration for the control female mice was higher than expected). The variability of the control female DOPAC

concentrations may also explain the lack of an increase in striatal dopamine turnover in the MPTP-treated female mice" (MRID 49122304, p. 342).

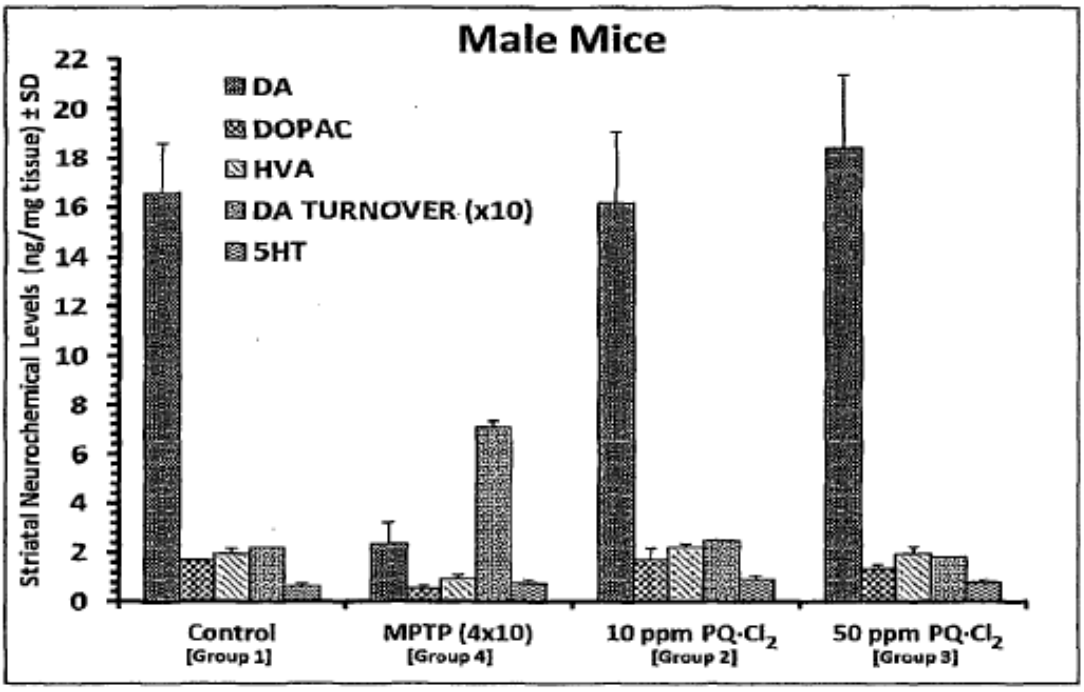
A summary of the results of the neurochemistry assessment is presented in Table 6 and Figures 2 and 3.

TABLE 6. Results of Neurochemistry Assessment (Subset III) [a, b]

Neurochemical	Dose level paraquat dichloride			
	Control	10 ppm	50 ppm	MPTP
Males				
Dopamine (ng/mg tissue)	16.55 ± 2.10	16.16 ± 2.93	18.43 ± 2.91	2.32** ± 0.89
DOPAC [b] (ng/mg tissue)	1.66 ± 0.09	1.70 ± 0.44	1.35 ± 0.15	0.53** ± 0.11
HVA (ng/mg tissue)	1.94 ± 0.19	2.16 ± 0.19	1.94 ± 0.30	0.93** ± 0.15
5HT [c] (ng/mg tissue)	0.62 ± 0.11	0.90* ± 0.17	0.76 ± 0.12	0.75 ± 0.13
Dopamine Turnover Rate [b]	0.220 ± 0.025	0.248 ± 0.066	0.180 ± 0.021	0.706** ± 0.269
Females				
Dopamine	16.75 ± 3.23	16.75 ± 2.36	20.14 ± 1.47	11.23 ± 4.63
DOPAC	3.25 ± 1.49	1.86 ± 0.48	1.66 ± 0.38	1.66 ± 0.59
HVA	1.91 ± 0.22	1.65** ± 0.07	1.87 ± 0.17	1.53* ± 0.18
5HT [a]	0.66 ± 0.09	0.73 ± 0.13	0.68 ± 0.07	0.72 ± 0.05
Dopamine Turnover Rate	0.330 ± 0.152	0.215 ± 0.056	0.176 ± 0.024	0.326 ± 0.137

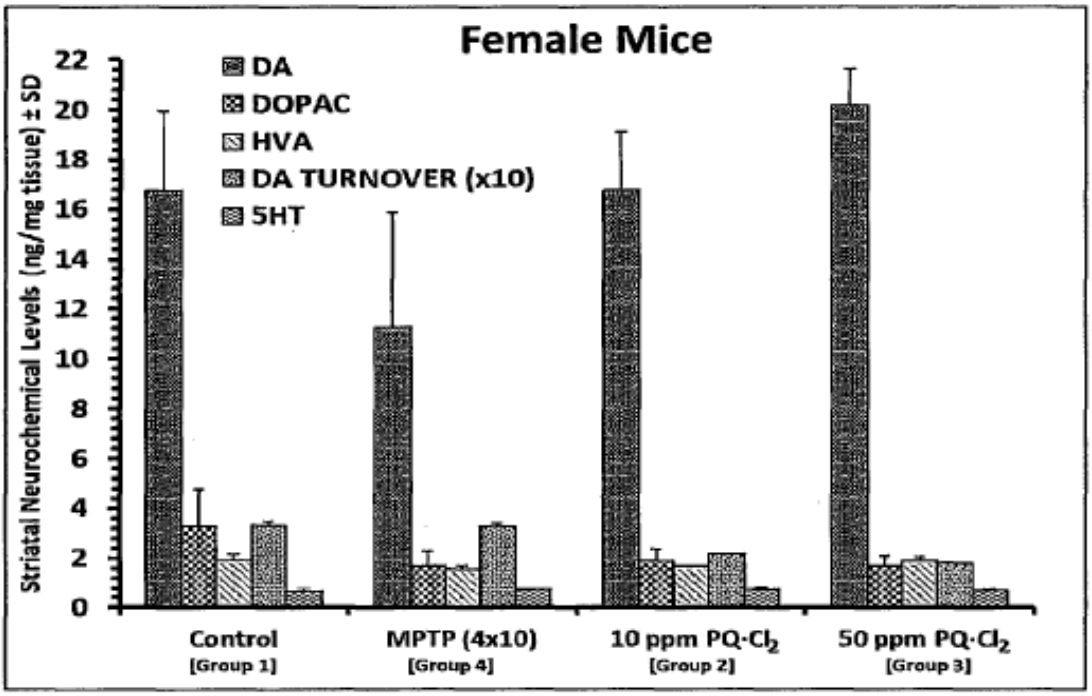
[a] Data taken from text tables in MRID 49122304, pp. 37, 337-339.
[b] Data not homogeneous using Bartlett's test
[c] Striatal serotonin concentrations were statistically analyzed as described in the protocol, but scientific interpretation of these data was precluded by the variability in the performance of the assay
Statistically different from controls: *p<0.05; **p<0.01

Figure 2. Striatal neurotransmitter and metabolite levels (ng/mg tissue) in C57BL/6J male mice after treatment with either paraquat dichloride or MPTP.



Reproduced from MRID 49122304, p. 340

Figure 3. Striatal neurotransmitter and metabolite levels (ng/mg tissue) in C57BL/6J female mice after treatment with either paraquat dichloride or MPTP.



Reproduced from MRID 49122304, p. 341

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

In mice fed 10 or 50 ppm paraquat dichloride, there were no effects on dopaminergic neurons as determined by the absence of any effect on striatal neurochemical concentrations (including dopamine, DOPAC, and HVA), the lack of neuronal, astrocyte, or microglial changes in the substantia nigra or the striatum, and the lack of effect on the number of TH^+, TH^-, or total neurons in the substantia nigra pars compacta. The effect of paraquat dichloride was limited to lower mean body weight gains for the 50 ppm paraquat dichloride group males during the first week of test diet administration.

Administration of MPTP resulted in a reduction in striatal dopamine concentrations for the MPTP-treated mice, relative to controls; the effect was greater for males than for females. There was decreased TH staining in the striatum of all MPTP-treated animals, and decreased TH staining in the substantia nigra of males. Furthermore, the number of TH^+ neurons and the contour volume of the substantia nigra pars compacta were decreased in males.

B. REVIEWER COMMENTS:

Treatment with 10 ppm or 50 ppm paraquat dichloride did not result in any observable clinical signs. Three males at 10 ppm and one male at 50 ppm were found dead, but the mortalities were not considered related to treatment because of a lack of dose-response and the lack of clinical signs and gross necropsy findings. One female at 50 ppm was euthanized following clinical signs of hunched posture and decreased defecation on the day of treatment and weight loss prior to euthanasia. Gross necropsy did not reveal the cause of moribundity; therefore, the death was not ascribed to treatment.

Males at 50 ppm had a transient, statistically significantly reduced body weight gain over the first week of treatment (-83% of controls). Reductions in mean absolute body weight observed in males at 50 ppm were not biologically significant, being within 5% of the control values. No effects on body weight were noted in males at 10 ppm or females at 10 or 50 ppm. No adverse effects on food consumption were noted. Although the reduced body weight gain in males at 50 ppm was transient, it was considered an adverse effect of treatment because it was not accompanied by a corresponding reduction in food consumption.

No changes were noted during gross necropsy, and treatment with up to 50 ppm paraquat dichloride did not affect brain weight, brain measurements, stereological evaluation, neuropathological assessment of brain sections, or neurochemical concentrations of dopamine or its metabolites DOPAC (dihydroxyphenylacetic acid) and HVA (homovanillic acid) in the striatal tissue of treated mice. Because no other adverse effects of treatment were observed, the transient reduction in body weight gain observed in males at 50 ppm during the first week of treatment was not considered adverse.

Male and female mice treated with MPTP exhibited reversible clinical signs including hunched posture, piloerection, hypoactivity, and/or tremors following one or more dose administrations. These clinical signs were not present a week following dosing. In the week following dosing, males and females exhibited weight loss which was attributed to treatment with MPTP. No definitive effects on food consumption were observed. Treatment with MPTP did not affect brain weights or measurements. A statistically significant decrease in TH^+ neurons and in the total contour volume was seen in male mice, but not female mice. In the striatal tissues from male mice treated with MPTP, marked decreases were observed in mean dopamine, DOPAC, and HVA concentrations with an associated increase in the mean dopamine turnover. The authors state that these findings are consistent with the known neurotoxicity of MPTP. The decreases in mean dopamine, DOPAC, and HVA concentrations observed for the striatal tissues obtained for the MPTP-treated female mice as compared to those for the control female mice were less than noted for the male mice.

Based on a transient decrease in body weight gain, the LOAEL was 50 ppm (10.2 mg/kg bw/day in males and 15.6 mg/kg bw/day in females), and the NOAEL was 10 ppm (1.7 mg/kg bw/day in males and 2.7 mg/kg bw/day in females).

C. STUDY DEFICIENCIES:

A major study deficiency was that the analytical data indicated that the mixing procedure was not adequate with % RSDs generally much greater than 10%. The variance between nominal and actual dosage to the animals was generally acceptable except for the 10 ppm diet formulations on the last sample day; reanalysis revealed a concentration within 16% of target. Stability analysis revealed ending concentrations much greater than the starting concentrations. Although a previous validation study reported acceptable 12 day stability data, the results from this study were not acceptable.

EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** July 16, 2014
Template version 09/11

TXR#: 0056764

DATA EVALUATION RECORD

STUDY TYPE: Range-Finding Feeding Study - Rabbit; Non-Guideline.

PC CODE: 061601 **DP BARCODES:** D409213

TEST MATERIAL (PURITY): Paraquat dichloride technical (32.32% a.i.)

SYNONYMS: YF6219

CITATION: Moxon, M. (1999) Paraquat dichloride - Dose range finding study in pregnant rabbits (final report). Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Laboratory report number CTL/T/2963, August 6, 1999. MRID 49009506. Unpublished. 55 pages.

SPONSOR: Syngenta Crop Protection, LLC, 410 Swing Road, Greensboro, North Carolina.

EXECUTIVE SUMMARY:

In a non-guideline range-finding feeding study (MRID 49009506), paraquat dichloride technical (32.32% a.i.; Batch no. D9485/072) was administered to ten time-mated female New Zealand White rabbits/group in feed at fixed dietary concentrations of 0, 35, 70, or 140 ppm from gestation day 8 through gestation day 21 to achieve nominal doses of 0, 2, 4 and 8 mg paraquat dichloride/kg/day, respectively. However the actual dose equated to 1, 2 and 4mg/kg/day rather than 2, 4 and 8mg/kg/day. There was little variation in dose received during the study for the 35ppm group but, in the 70 and 140ppm groups, daily variation occurred with the changes in food consumption. Dose selection was based on preliminary range-finding studies (MRID 49009507, 49009508). The purpose of this study was to investigate the effects of dietary administration of paraquat dichloride on the pregnant New Zealand White rabbit and to determine appropriate dose levels for a developmental toxicity study. Previous developmental studies in rabbits (CTL Report Numbers CTL/P/2749 and CTL/P/2763 – not available for review) administering paraquat dichloride by oral gavage at dose levels of 1-2 mg/kg/day resulted in overt maternal and fetal toxicity which made it difficult to assess the developmental toxicity because of the low number of litters available for external examination.

Rabbits were examined daily for clinical conditions. Body weights and food consumption were recorded daily. Plasma creatinine and basal blood urea nitrogen were measured on day 18. The animals were terminated on day 30 of gestation. The animals were examined macroscopically. Number of corpora lutea, gravid uterus weight, implantations, live fetuses, early and late intra-uterine deaths and fetal weight were all recorded. Fetuses were examined for external abnormalities only.

Two animals from the 140 ppm group were sacrificed on day19 and another animal on Day 18 due to weight loss, negligible food consumption and few feces. Post mortem examination revealed abnormal stomach contents in 2 of the 3 animals. There was a slightly higher incidence of animals with reduced feces in the 70 and 140 ppm groups. No other treatment related clinical conditions were reported. Excluding the three animals from the 140 ppm group that were sacrificed prior to study termination, there was no effect on body weights or food consumption.

There was no difference in plasma creatine and basal blood nitrogen among groups except for the 140 ppm group where an increase in plasma creatine was seen when the 3 animals that were sacrificed prior to study termination were included in the calculation of the mean.

Three of the 10 animals in the 70 ppm group had abnormal stomach contents and 2 had a sloughed glandular mucosa. These findings were not seen in the animals given 140 ppm which survived to scheduled termination. However, one animal given 140 ppm did have areas of ulceration in the stomach (and 2 of the 3 animals which died during the study had abnormal stomach contents).

Three of the 10 animals in the 70 ppm group had abnormal stomach contents and 2 had a sloughed glandular mucosa. These findings were not seen in the animals given 140 ppm which survived to scheduled termination. However, one animal given 140 ppm did have areas of ulceration in the stomach (and 2 of the 3 animals which died during the study had abnormal stomach contents).

There was no evidence for an adverse effect of paraquat dichloride on the number, growth, external abnormalities or survival of the fetuses in utero.

The reviewer finds this range-finding study to be inconclusive. Actual dosage to the animals was variable. The diet was not analyzed for homogeneity or stability. No individual animal data was provided. The developmental segment of the study did not provide all parameters. A lowest observed adverse effect level (LOAEL) could not be established from this study. Equivocal reduction in body weight and food consumption was seen at the 70 and 140 ppm group animals along with reduced fecal excretion. Individual clinical signs, body weight, food consumption, cesarean section and gross pathology data were not provided. The study was not conducted in compliance with the GLP principles. This study is classified **Unacceptable/non-Guideline.**

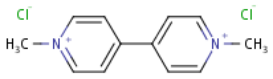
COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. According to the GLP Compliance Statement, the study was not conducted in compliance with OECD Principles of Good Laboratory Practice (1997). Quality Assurance statement was not provided. The study included a Statement of Authentication signed by the study director that included the following assertion: The data described in this report have not been subjected to audit by the Laboratory's Quality Assurance Unit but are derived from a study which is considered to meet the principles of Good Laboratory Practice.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Paraquat dichloride

Description:	Technical material, YF6219; dark brown liquid
Batch #:	D9485/072 [Bottle No. 128621]
Purity:	32.32% a.i.
Compound stability:	Confirmed March 20, 1998, stability confirmed by re-analysis after dosing had ended
CAS #of TGAI:	1910-42-5
Structure:	



2. Vehicle and/or positive control: The test material was incorporated into the diet; deionized water was used to help with mixing and pelleting. A positive control was not used in the study.

3. Test animals:

Species:	Rabbit (females, only)	
Strain:	New Zealand White	
Age/weight at study initiation:	Age not reported/body weights within a range of 3.3-4.0 kg	
Source:	Harlan UK Limited	
Housing:	Individually in "mobile rabbit units"	
Diet:	Harlan Teklad 9603 TRBDiet, *ad libitum*	
Water:	Municipal tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	17±3°C
	Humidity:	30-70%, with an excursion to 83.5% on one occasion
	Air changes:	15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days	

B. PROCEDURES AND STUDY DESIGN

1. Purpose: The purpose of this study was to investigate the effects of dietary administration of paraquat dichloride on the pregnant New Zealand White rabbit and to determine appropriate dose levels for a developmental toxicity study.

2. In life dates: Start: October 6, 1997; End: November 5, 1997.

3. Animal Assignment: Animal assignment is given in Table 1. The study was divided into ten replicates (randomized blocks), each containing one cage/animal per experimental group.

Computer-generated, random number permutations were used to allocate the cages within each replicate to an experimental group.

TABLE 1. Animal assignment [a]

Group	dietary concentration of paraquat dichloride (ppm)	Target dose (mg/kg bw/day)	Calculated dose (mg/kg bw/day)*	Number assigned
1	0	0	0	10
2	35	2	1.2 (1.0-1.5)	10
3	70	4	2.2 (1.4-2.9)	10
4	140	8	3.0 (1.3-5.7)	10

[a] Data taken from text table, p. 16, MRID 49009506 and page 15.
* Mean (range)

4. **Dose selection rationale:** Dose levels were selected based on the results from a range-finding study in non-pregnant female rabbits in which dietary administration of paraquat dichloride at concentrations of levels of 80, 179, and 332 ppm for up to 7 days resulted in dose-related effects on body weight gain and food consumption (MRID 49009507). In another range-finding study in non-pregnant rabbits administered paraquat dichloride at concentrations of levels of 228 or 317 ppm for 13 days resulted in similar effects and reduced fecal excretion (49009508).

5. **Diet preparation and analysis:** The calculated concentrations (ppm) were based on a 3.5kg rabbit eating 200 g diet per day. One batch of diet formulation was prepared per concentration. For each dietary formulation, a premix was made by dispensing the appropriate amount of paraquat dichloride (corrected for purity) with 12 mL of deionized water into the grinder bowl, using an additional 2 x 12 mL volumes of deionized water to wash the sample bottle into the grinder bowl, adding a 1000-g quantity of the basal diet, and grinding the contents of the bowl until the test substance was thoroughly mixed into the diet, at which point the premix was passed through a 1.0 mm sieve, and any lumps were ground and sieved again. The premix was then transferred to a mixer and left to mix at minimum speed for 30 minutes. The premix was added to the rest of the control diet and mixed for 4 minutes using an automated mixer, and then was transferred with approximately 11% water to a different mixer and mixed for 6 minutes. The diet was then pelleted (3-mm size nominally), using a California pellet mill. The resultant pellets were dried in an autoclave at approximately 52°C for approximately 1 hour 45 minutes, cooled, and dispensed into color-coded plastic containers. The diets were stored at room temperature until used. Diets were not analyzed for test material concentration. Homogeneity and stability were not evaluated.

   Animals were given the treated diet from gestation Day 8 through gestation Day 21.

Results:
Concentration analysis: Diets were not analyzed for test material concentration.

In the absence of stability and homogeneity data, it is unknown whether the mixing procedure was adequate or whether the variance between nominal and actual dosage to the study animals was acceptable.

C. METHODS:

1. **Mortality and clinical observations:** Detailed clinical observations were recorded daily and, where appropriate, at the same time that the animals were weighed.

2. **Body weight:** The animals were weighed on the day following delivery to the testing facility, on days 4, 8-21 (inclusive), 25 and 30 of gestation.

3. **Food consumption:** Food consumption was recorded beginning on Day 4. Food wastage was recorded.

4. **Test material intake:** Each animal's received dose was calculated daily as follows:

$$\text{Dose received (mg/kg bw/day)} = \frac{\text{dietary concentration (ppm) x food consumption (g)}}{\text{average body weight (g)overdays n and n + 1}}$$

5. **Blood Sampling:** On day 18, 2ml of blood was taken from the lateral ear vein of each rabbit and collected in tubes containing lithium heparin as anticoagulant. The samples were analyzed for the determination of plasma creatinine and basal blood urea nitrogen.

5. **Sacrifice and pathology:** The animals were killed on day 30 via intravenous injection of pentoparbitone sodium solution. All were subjected to a gross necropsy involving external observation and examination of the thoracic and abdominal viscera. The pregnancy status of each animal was determined. The uterine contents and ovaries were examined and the following parameters were recorded:
   - Number of corpora lutea in the ovaries
   - Gravid uterus weight
   - Litter data: number and position of implantations, number of live fetuses, number of intra-uterine deaths (early and late)
   - Fetal weights and external abnormalities including oral cavity.

No weights were recorded or fetal examinations for animals that died prior to scheduled termination.

D. DATA ANALYSIS:

1. **Statistical analyses:** Data relating to animals which were non-pregnant were excluded. Maternal bodyweight (including and excluding intercurrent deaths) during the dosing period were considered by analysis of covariance on initial (day 8) bodyweight.

   Maternal food consumption (including and excluding intercurrent deaths) during the dosing and post dosing periods, the numbers of implantations and live fetuses per female, gravid uterus weight, litter weight and mean fetal weights per litter were considered by analysis of variance.

   Maternal performance data were considered by Fisher's Exact Test.

$$\%\ \text{pre} - \text{implantation loss} = \left(\frac{\text{number of corpora lutea} - \text{number of implantations}}{\text{number of corpora lutea}}\right) x\ 100$$

$$\%\ \text{post} - \text{implantation loss} = \left(\frac{\text{number of implantations} - \text{number of live fetuses}}{\text{number of implantations}}\right) x\ 100$$

Percentage Implantation losses were analyzed by analysis of variance following double arcsine transformations of Freeman and Tukey (1950). The proportion of fetuses and the proportion of litters affected were considered by Fisher's Exact Test.

All analysis were carried out in a 1989 SAS program.

2. Historical control data: Historical control data were not provided.

II. RESULTS:

A. MORTALITY AND CLINICAL OBSERVATIONS: Two animals from the 140 ppm group were sacrificed on day19 and another animal on Day 18 due to weight loss, negligible food consumption and few feces. Post mortem examination revealed abnormal stomach contents in 2 of the 3 animals. There was a slightly higher incidence of animals producing feces in the 70 and 140 ppm groups. No other treatment related clinical conditions were reported.

B. BODY WEIGHT: Selected body weight data are given in Table 2. Excluding the three animals from the 140 ppm group that were sacrificed prior to study termination, there was no effect on body weights.

TABLE 2. Mean (±SD) body weight data (g) [a] in rabbits administered paraquat dichloride

Parameter / Study day or interval	Dietary concentration in ppm [group size, n]			
	0 (10)	35 (9)	70 (10)	140 (7)
Body weights excluding intercurrent deaths				
Absolute body weight: Day 8	4083±260	4069±245	4158±115	4051±333
Day 11	4109±254	4050±208	4158±149	3954±327**
Day 18	4170±254	4157±277	4224±138	4056±405
Day 19	4188±239	4154±278	4244±151	4066±377
Day 21	4151±279	4177±304	4229±150	4111±370
Day 30	4278±303	4336±299	4287±194	4291±432
Body weights including intercurrent deaths				
	0 (10)	**35 (9)**	**70 (10)**	**140 (10)**
Absolute body weight: Day 8	4083±260	4069±245	4158±115	4061±293
Day 11	4109±254	4050±208	4158±149	3935±290**
Day 18	4170±254	4157±277	4224±138	3913±418**
Day 19	4188±239	4154±278	4244±151	3939±422**

[a] Data taken from Table 4, pp. 35-38, MRID 49009506.

** Significant at p<0.01.

C. FOOD CONSUMPTION: Selected food consumption data are given in Table 3. There was a marked reduction in food consumption in the 140 ppm group due to three animals that were severely affected. Also, there was evidence for an effect of 70 ppm paraquat dichloride on food consumption between days 11 and 14. However, there was no statistically significant differences from control for the 35 or 70 ppm groups during the treatment period.

TABLE 3. Mean (±SD) food consumption (g/animal/day) [a] in rabbits administered paraquat dichloride				
Study day or interval	**Dietary concentration in ppm [group size, n]**			
	0 (10)	**35 (9)**	**70 (10)**	**140 (7)**
Food consumption excluding intercurrent deaths				
Day 8	159±53	154±51	168±50	139±70
Day 9	163±27	147±49	147±56	93±42**
Day 10	161±44	143±38	173±55	46±34**
Day 11	142±42	154±48	138±61	58±41**
Day 12	128±49	133±55	117±50	73±50
Day 15	129±51	124±74	97±42	106±73
Day 19	173±49	168±70	152±67	165±67
Day 21-25	177±91	139±24	119±40*	153±24
Food consumption including intercurrent deaths				
	0 (10)	**35 (9)**	**70 (10)**	**140 (10)**
Day 8	159±53	154±51	168±50	132±59
Day 9	163±27	147±49	147±56	68±54**
Day 10	161±44	143±38	173±55	36±33**
Day 11	142±42	154±48	138±61	42±48**
Day 12	128±49	133±55	117±50	52±53**
Day 13	130±41	115±56	95±44	58±66**
Day 18	167±43	163±86	148±65	112±74

[a] Data taken from Table 4, pp. 39-42, MRID 49009506.

** Significant at $p<0.01$.

D. COMPOUND INTAKE: Compound intake of the treated animals in mg/kg bw/day is summarized in Table 4.

TABLE 4. Paraquat dichloride doses, including intercurrent deaths (mg/kg bw/day) [a]			
Study Day	**Dietary concentration in ppm [group size, n]**		
	35	70	140
Day 8	1.3	2.8	4.6
Day 9	1.2	2.5	2.4
Day 10	1.3	2.9	1.3
Day 11	1.3	2.3	1.4
Day 12	1.1	2.0	1.8
Day 13	1.0	1.6	2.0
Day 14	1.0	1.4	2.1
Day 15	1.0	1.6	2.6
Day 16	1.2	2.1	3.0
Day 17	1.3	2.4	3.0
Day 18	1.4	2.4	3.9
Day 19	1.4	2.5	5.7
Day 20	1.5	2.4	5.4
Mean	**1.2**	**2.2**	**3.0**

[a] Data taken from Table 1, p. 32, MRID 49009506.

E. BLOOD CLINICAL CHEMISTRY: There was no difference in plasma creatine and basal blood nitrogen among groups except for the 140 ppm group where an increase in plasma creatine was seen when the 3 animals that were sacrificed prior to study termination were included in the calculation of the mean.

F. GROSS PATHOLOGY: Three of the 10 animals in the 70 ppm group had abnormal stomach contents and 2 had a sloughed glandular mucosa. These findings were not seen in the animals given 140 ppm which survived to scheduled termination. However, one animal given 140 ppm did have areas of ulceration in the stomach (and 2 of the 3 animals which died during the study had abnormal stomach contents).

F. CESAREAN SECTION DATA: There was no evidence for an adverse effect of paraquat dichloride on the number, growth or survival of the fetuses in utero (Table 5).

B. DEVELOPMENTAL TOXICITY:

1. External examination: Four fetuses, one from each treatment group (including the control group), were found to have flexed forepaws. In addition, the fetus in the 140ppm group had an encephalocele. Flexed forepaws were seen in all groups including the control and the encephalocele was of single incidence only. These fetal abnormalities were therefore judged to be incidental to treatment.

2. Visceral examination: Not done.

3. Skeletal examination: Not done.

TABLE 5 Cesarean section observations [a] in rabbits administered paraquat dichloride				
Observation	**Dose (mg/kg bw/day)**			
	0	**LDT**	**MDT**	**HDT**
# Animals assigned (mated)	10	10	10	10
# Animals pregnant	10	9	10	10
Pregnancy rate (%)	100	90	100	100
# Nonpregnant	0	1	0	0
Maternal wastage				
No. died	0	0	0	3
No. died pregnant	0	0	0	3
No. died nonpregnant	0	0	0	0
No. aborted	0	0	0	0
No. Premature delivery	0	0	0	0
Live fetuses in utero at termination	10/10	9/10	10/10	7/10
Total No. corpora lutea **Corpora lutea/dam**	- 10.3±1.8	- 11.1±1.1	- 10.9±2.2	- 10.7±1.8
Total No. implantations **(Implantations/dam)**	- 8.10±3.35	- 10.22±1.39	- 9.60±3.303	- 9.43±3.41
Total No. litters				
Total No. live fetuses **(Live fetuses/dam)**	- 6.70±2.91	- 9.56±1.59*	- 8.70±2.79	- 8.57±3.05
Total No. dead fetuses **(Dead fetuses/dam)**	-	-	-	-
Total No. resorptions	-	-	-	-
Early	-	-	-	-
Late	-	-	-	-
Resorptions/dam				
Early **Prop. of litters affected**	10.8±11.5 (6/10)	1.2±3.7** (1/9)	3.7±4.8* (4/10)	1.2±3.1** (1/7)
Late **Prop. of litters affected**	4.2±7.6 (3/10)	5.4±7.0 (4/9)	4.9±5.2 (5/10)	6.2±9.3 (3/7)
Litters with total resorptions				
Mean gravid uterus weight (g)	447±147	595±59*	571±155	530±158
Mean litter weight (g)	290±106	392±46	328±109	342±113
Mean fetal weight (g)	46.0±7.5	41.4±3.6	38.8±6.9*	41.6±6.9
Males	-	-	-	-
Females	-	-	-	-
Sex ratio (% male)	-	-	-	-
Preimplantation loss (%) **Prop. of litters affected**	22.9±29.6 7/10	7.9±10.3* 4/9	14.9±17.8 8/10	15.3±25.1 5/7
Postimplantation loss (%) **Prop. of litters affected**	14.9±13.1 8/10	6.7±6.9* 5/9	8.6±8.5 6/10	7.4±12.2 3/7
[a] Data taken from Table 2 p. 33 and Table 8, p. 48-49 MRID 49009506.				

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The purpose of this study was to evaluate the effects in the rabbit of nominal dose levels of 2, 4 and 8mg paraquat dichloride/kg/day by incorporating the paraquat dichloride in the diet at fixed concentrations of 35, 70 and 140ppm respectively. From calculation of the actual dose received it is clear that the nominal dose levels were not achieved and equated to 1, 2 and 4mg/kg/day rather than 2, 4 and 8mg/kg/day. There was little variation in dose received during the study for the 35 ppm group but, in the 70 and 140 ppm groups, daily variation occurred with the changes in food consumption. Unequivocal evidence of maternal toxicity was not obtained. There was an effect of 70 and 140ppm paraquat dichloride on bodyweight and food consumption which resulted in reduced fecal output but whether this was a direct or indirect effect of paraquat dichloride was not established.

B. REVIEWER COMMENTS: The reviewer finds this study to be inconclusive. Actual dosage to the animals was variable. The diet was not analyzed for homogeneity or stability. No individual animal data was provided. The developmental segment of the study did not provide all parameters. A lowest observed adverse effect level (LOAEL) could not be established from this study. Equivocal reduction in body weight and food consumption was seen at the 70 and 140 ppm group animals along with reduced fecal excretion. Individual clinical signs, body weight, food consumption, and gross pathology data were not provided. The study was not conducted in compliance with the GLP principles. This study is classified **Unacceptable/non-Guideline.**

C. STUDY DEFICIENCIES: The following deficiencies were noted:

- The study was not conducted in compliance with the Principles of Good Laboratory Practice.
- No Quality Assurance statement was provided.
- Individual clinical signs, body weight, food consumption, and gross pathology data were not provided.
- Stability and homogeneity of the prepared diet formulations were not evaluated.
- Cesarean section observations are incomplete.